

**THE PRODUCTION AND PERCEPTION OF FACIAL EXPRESSIONS BY INFANTS  
AT HIGH-RISK FOR AN AUTISM SPECTRUM DISORDER**

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Individuals with autism spectrum disorder (ASD) display atypical production and perception of emotion. Infant sibling studies, longitudinal assessments of infants with (i.e., high risk) and without (i.e., low-risk) an older sibling with ASD, have reported that the production of social smiles may indicate ASD as early as 12 months of age. Still, little is known about the nature of HR and low-risk (LR) infants' emotion development prior to 12 months. The current study explores HR and LR infants' facial expression production and perception at 6 and 11 months of age. The social signals of emotion produced by 26 HR 6-month-olds, 24 LR 6-month-olds, 24 HR 11-month-olds, and 33 LR 11-month-olds were measured in the context of face-to-face interaction with their mothers. Results indicated that increased positive affect at 6 months may characterize HR infants later diagnosed with ASD (HR-ASD infants), while increased looking to mother at 6 months and increased positive affect at 11 months may characterize HR infants with no known diagnosis of ASD (HR-no ASD). Eye-tracking methods were utilized to measure visual attention to smile/neutral face pairings displayed by 31 HR 6-month-olds, 28 LR 6-month-olds, 37 HR 11-month-olds, and 32 LR 11-month-olds. Results revealed that increased visual

attention to the whole stimulus and the internal features of the face at 6 months may characterize HR infants as a whole (i.e., HR-no ASD and HR-ASD). Taken together, these findings suggest that emotion production and perception help define the early phenotype of HR infants. Increased positive affect and looking to affective stimuli observed in the HR sample are discussed as possible indicators of arousal regulation and visual disengagement difficulty. All findings are discussed from the theoretical framework of transactional models of child development and experience-expectant model of emotion development.

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## **1.0 INTRODUCTION**

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders defined by deficits in social communication, social reciprocity, and the presence of stereotyped behavior and/or interests (American Psychiatric Association, 2000, 2013). Although conceptualized as genetically-based and present from birth (Kanner, 1943; Nicholson & Szatmari, 2003), clinically relevant levels of the behavioral features of ASD cannot be reliably detected until twelve months of age (e.g., Luyster et al., 2009). Determining the qualities of ASD that emerge during the first year of life is of paramount importance in the field of autism. These discoveries would improve early screening, lead to novel early treatments, and inform studies of the underlying genetic and neurological mechanisms of ASD. The current study focuses on social-emotional development, specifically the production and perception of facial expressions of emotion, as potentially important to the early development of infants at risk for ASD.

In order to examine how ASD emerges during the first year of life, researchers currently utilize the prospective, infant sibling method. This method is based on knowledge of the sibling recurrence risk of ASD (i.e., 18.7 percent; Ozonoff et al., 2011) and longitudinally assesses the early development of infants with (i.e., high-risk) and without (i.e., low-risk) an older sibling with ASD. These studies define characteristics that emerge during the first years of life to mark high-risk (HR) status and predict an ASD diagnosis (see Rogers, 2009 for a review).

Contrary to initial predictions of the research community, infant sibling studies suggest that behavioral markers once considered to be optimal candidates for early identification of ASD (e.g., eye contact, social smiling, vocalizations measured in an interaction context) have limited predictive value prior to twelve months of age (e.g., Bedford et al., 2012; Ozonoff, et al., 2010; Rozga, et al., 2011; Zwaigenbaum et al., 2005). In contrast, investigations that utilize brain imaging and neurocognitive measures to examine brain structure and function, such as diffusion tensor imaging (Wolff et al., 2012) and electroencephalography (e.g., Bosl, Tierney, Tager-Flusberg & Nelson, 2011), have consistently reported finding markers that are predictive of ASD prior to one year of age. These observations have lead some researchers to hypothesize that the neurocognitive phenotype of ASD may emerge first and over time contribute to the behavioral characteristics that traditionally define the disorder spectrum (e.g., Hutman, 2013).

Visual attention has been suggested as a neurocognitive mechanism that may mediate and/or modulate the relationship between early emerging atypical neurological functioning and atypical observable behavior in ASD (e.g., Elsabbagh & Johnson, 2010; Chawarska, Macari, & Shic, 2013). It appears that elementary aspects of visual attention, measured via looking and eye-tracking paradigms, predict to later ASD diagnosis when assessed during the first year (e.g., Chawarska et al., 2013; Zwaigenbaum et al., 2005) to 18 months of life (e.g., Elsabbagh et al., 2013). Research also indicates that typical developments in visual attention reflect the intertwined development of perceptual and neurological systems (Colombo, 2001; Johnson, 2000) and that visual attention is important to typical development of observable social, emotional and communicative behaviors from infancy (e.g., Rochat & Striano, 1999). Therefore, it is possible that atypical visual attention in infants later diagnosed with ASD a) reflects early

atypical neurological function and b) contributes to the observable, behavioral, socio-emotional atypicalities that currently define ASD.

Infant sibling studies of emotion perception and production are well-suited to assessing the developmental course of and relation between visual attention and observable, social-emotional behavior as markers of HR and ASD during the first year of life. Such investigations are supported by theory and empirical data. Theoretical models of emotional development and empirical data suggest that typical infants' neural and perceptual systems are tuned to social signals of emotion during the first year of life based on naturalistic experience (e.g., see Leppanen & Nelson, 2009 for a review). The transactions that occur within a social context, such as engagement with a parent in a social action game, shape infants' emotional development (e.g., Sameroff, 1975, 2009). It is also widely noted that individuals with an ASD diagnosis display atypical production and perception of facial expressions (Harms, Martin & Wallace, 2010; Hobson, 2005). In addition, individuals with ASD have life-long impairments in, and therefore atypical experience with, affective behavior and reciprocity (Hobson, 2005). In fact, infant sibling studies have recently shown atypical emotion production (i.e., social smiling) to be predictive of an ASD as early as twelve months of age (Ozonoff et al., 2010; Rozga et al., 2011). If experience with the social signals of emotion typically tunes emotion perception from the first year of life *and* individuals with ASD are known to have atypical experience with the social signals of emotion from twelve months of age onward, it is possible that atypical production and processing of emotion begins prior to twelve months of age.

The current study investigates the production and perception of facial expressions in HR and low-risk (LR) infants at 6 and 11 months of age. The overarching goals of this study are to further define the very early emotional development of HR infants and infants with ASD by a)



cataloguing the quality of the early emotion production and perception, b) describing the timing of emergence of emotion production and perception abilities, c) describing the effect of context (i.e., mother's behavior, interaction type, facial expression intensity) on these emerging abilities, and d) commenting on potential interplay between emotion perception and production within this HR sample during the first year of life. It is hypothesized that visual attention to facial expressions of emotion, in the context of an eye-tracking paradigm and mother-infant interaction, may differentiate HR from LR infants *and* indicate ASD diagnosis as early as 6 months of age. It is also predicted that while infant's production of facial expressions may also distinguish HR and LR infants at 6 and 11 months, these observable behaviors will not be indicative of ASD until 11 months of age.

To provide further rationale for the current study aims and predictions, a theoretical framework and empirical support are presented below. First, Leppanen and Nelson's (2009) model of experience-expectant emotional development and Sameroff's (1975, 2009) transactional theory of development are described, as they present a theoretical frame for the current study. Next, an empirical basis for the current infant sibling study of emotion perception and production is developed. To do this, studies that document emotion production and perception atypicalities in individuals with an ASD are described, followed by a description of empirical studies that document the typical development of emotion production and perception from infancy. Finally, findings from the infant sibling investigations that have assessed emotion production and perception as markers of HR and predictors of ASD diagnosis are presented. These studies provide the basis for specific study aims and hypotheses.

## 1.1 A MODEL OF EXPERIENCE-EXPECTANT EMOTION DEVELOPMENT

Leppanen and Nelson's (2009) model of infants' emotional development proposes that development in this domain requires a dynamic integration of neural, perceptual and behavioral systems across time and with experience. The general premise behind experience-expectant models is that genetically-determined neural maturation progresses in a way that matches in time with, and in interaction with, experiences, activity, or adaptive tasks (Greenough, Blake, & Wallace, 1987). This idea provides the basis of Leppanen and Nelson's (2009) model. In this model it is proposed that experience with social signals of emotion is necessary to tune emotion-specific neural and perceptual networks during the first years of life. General experience-expectant theories provide plausible explanations for infant sibling findings regarding the timing of the emergence of atypicalities across neural, perceptual, and behavioral systems (e.g., Hutman, 2013). Emotion-specific experience-expectant theories provide a theoretical guide for the current study of emotional development in at-risk infants.

*Experience-expectant* mechanisms are defined as neurological and perceptual mechanisms that are biased toward, or "expectant" of salient signals from the first year of life. These systems are thought to be pre-wired to respond to emotion signals. Evidence for the pre-wired nature of these systems comes from early maturation of emotion-related neural circuits, functional coupling of these structures with visual-representation areas, and infants bias to attend to emotionally salient (e.g., fearful faces) over neutral facial expressions (Leppanen & Nelson, 2009). It is proposed that the predisposition of neural and perceptual systems to process social signals of emotion is fine-tuned by experience, particularly species-typical exposure to facial expressions.

*Experience-dependent* processes are the mechanism by which individual-specific experiences, such as the frequency and intensity of certain facial expressions in the rearing environment, shape emotional development. While *experience-expectant* processes are thought to be pre-wired and shape how neural and perceptual systems become roughly specified toward species-typical emotional expressions, *experience-dependent* processes are a mechanism by which individual-specific experience can shift this neurocognitive tuning to emotional signals. Leppanen and Nelson (2009) suggest that the experience-dependent process may explain heightened sensitivity or augmented perceptual processing of particular emotional stimuli in children with atypical emotional experience. For example, children who experience early institutional rearing display atypical emotion production (e.g., indiscriminate friendliness; Sloutsky, 1997), arousal regulation (Tottenham et al., 2010), and emotion perception (e.g., atypical electrophysiological response to facial expressions; Parker & Nelson, 2005). These atypicalities in emotion development are assumed to be shaped by these children's unique emotional experience (Johnson, 2000).

Leppanen and Nelson (2009) make the broad suggestion that dysfunction of experience-expectant and -dependent processes are precursors to social disorder. It has long been assumed that the social impairments of individuals with ASD lead to a unique social experience which has cascading and continuous effect on socio-emotional development. This describes the impact of atypical experience, and therefore describes an *experience-dependent* process. More recently, infant sibling researchers have begun to suggest an early disruption of *experience-expectant* mechanisms may contribute to social disability in ASD from the first years of life (Chawarska et al., 2013; Hutman, 2013; Jones & Klin, 2013). Infant sibling researchers have consistently reported neurocognitive anomalies as early as 6 months of age, prior to the emergence of

behaviorally evident social disability, in infants later diagnosed with ASD (e.g., Hutman, 2013). These anomalies appear in neural and perceptual systems, the experience-expectant systems that Leppanen and Nelson (2009) describe. If neural and perceptual systems are functioning atypically from the first year of life, this dysfunction could influence social experience and the development of social-emotional behavior. The current study investigates both emotion perception and production in an attempt to understand the relation between these developing systems in infants at risk for ASD.

## **1.2 DEVELOPMENT AS A TRANSACTIONAL PROCESS**

Lepannen and Nelson (2009) provide a conceptual framework for considering the bidirectional influence of neurocognitive abilities and social experience/behavior on an individual's trajectory of socio-emotional developmental trajectory. Transactional models of development emphasize the bidirectional effects that exist between an individual and the social context. Research designed from a transactional framework considers the parent a part of the social context influencing a child's development, but also considers that variability in the larger social interaction context (e.g., the type of caregiver-infant social game being played) contributes to inter-individual variability in the expression of social behavior (Lerner, 1991).

Studies of facial expression production and perception in ASD are not often designed from a transactional perspective. Few infant sibling studies of emotion development consider how features of the social context (e.g., the social-emotional behavior of the interaction partner in a face-to-face paradigm, the type of interaction, the intensity of facial expressions) influence the perception or expression of emotion, and how this may impact conclusions regarding typical

versus atypical developmental course. The current infant sibling study considers the relation between a) systems developing within infant siblings (i.e., perceptual and behavioral) and b) the individual's behavior and the social context (i.e., the mother's affective behavior, the social game type, intensity of facial expression) as being important to understanding the emotion development of infants at risk for ASD.

### **1.3 FACIAL EXPRESSION PRODUCTION AND PERCEPTION IN ASD**

Although there are still many questions that remain unanswered regarding the emergence of emotion production and perception difficulties in ASD, it is widely acknowledged that individuals with an ASD diagnosis atypically use facial expressions in the context of social interaction. The *Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> Edition* includes “marked impairment in the use of . . . facial expression” as a core diagnostic criteria (American Psychiatric Association, 2000). The newly published *Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> Edition* continues to include “deficits in social-emotional reciprocity, for example . . . reduced sharing of . . . emotions, or affect” as part of diagnostic criteria for ASD (American Psychiatric Association, 2013). Standardized diagnostic measures, considered the “gold-standard” for ASD diagnosis from 18 months of age onward, also confer diagnoses along the autism spectrum based, in part, on observing the range of facial expressions a child directs to others (Lord et al., 2000; Luyster et al., 2009).

The inclusion of emotion-specific deficits in diagnostic criteria and assessment has developed based on numerous studies showing atypical affective responses to socio-emotional stimuli through the life course. Studies utilizing parent report and observation of naturalistic

interaction indicate that toddlers and school-age children with autism display less positive and more negative affect (e.g., Capps, Kasari, Yirmiya, & Sigman, 1993; Snow, Hertzog, & Shapiro, 1987; Yirmiya, Kasari, Sigman, & Mundy, 1989), combine smiles with eye contact less often, and respond to their mother's smiles less often (Dawson, Hill, Spencer, Galpert, & Watson, 1990) than developmentally delayed or typical developing comparison groups. As individuals with ASD get older they display more negative affect and appear more emotionally labile than their typically developing and developmentally delayed peers (e.g., Bradley & Isaacs, 2006; Capps et al., 1993; White, Oswald, Ollendick, & Scahill, 2009). Research also suggests that displays of atypical facial expressions continue to characterize ASD into adulthood (e.g., MacDonald et al., 1989).

In addition to atypical use of facial expressions, individuals with ASD demonstrate impaired recognition and atypical processing of facial expressions (see Harms et al., 2010, for a review). Behavioral studies have shown that recognition of prototypical, high-intensity facial expressions of emotion, or facial expressions exhibiting basic emotions (e.g., fear) may be intact with difficulty in emotion recognition existing only for low-intensity, subtle, or complex (e.g., arrogance) facial expressions (e.g., Rutherford & Towns, 2008; Smith, Montagne, Perrett, Gill, & Gallagher, 2010). For individuals with ASD, intact behavioral performance (i.e., accuracy) on facial expression recognition tasks does not necessarily indicate typical *processing* at a neural and perceptual level. Nearly all studies of facial expression processing using neurocognitive methods (e.g., eye-tracking, neuroimaging) note significant differences between ASD and control groups, even when behavioral performance is intact (Mazefsky, Pelphrey, & Dahl, 2012). For instance, eye-tracking studies have found that compared to typically developing controls, individuals with ASD spend more time looking at the outer (e.g. hair) than inner (e.g., eyes,

nose, and mouth) regions of the face and more time looking at the mouth than eye regions of the face (e.g., Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Pelphrey et al., 2002). Researchers have suggested that individuals with ASD may use alternate, compensatory strategies to process facial expressions (e.g., see Pelphrey, Adolphs, & Morris, 2004 for a review).

Although it has been suggested that there is a relationship between atypical processing of facial expressions and deficits in social communication (e.g., García-Villamizar, Rojahn, Zaja, & Jodra, 2010; Pelphrey et al., 2002), and empirical evidence indicates a relationship between face processing and social impairments in ASD (e.g., Klin et al., 2002; McPartland, Webb, Keehn, & Dawson, 2011), it is not yet clear how these aspects of emotional development emerge together or relate to one another during the first years of life in ASD. Studies of typically developing infants suggest how emotion perception and production *typically* emerge in tandem during the first year of life. These studies provide a methodological framework and empirical background for infant sibling studies of emotion development.

#### **1.4 FACIAL EXPRESSION PRODUCTION AND PERCEPTION IN TYPICAL INFANCY**

Typically developing infants' early production of facial expressions is characteristically observed in mother-infant interaction contexts (e.g., face-to-face interaction, peek-a-boo; Fogel et al., 1997). Infants' produce smiles as neonates, but smiles are not thought of as containing communicative content until social smiling (e.g., smiling paired with visual engagement) emerges between 4 and 6 weeks of age (Messinger & Fogel, 2007). It has been suggested that cycles of visually-directed smiling and visual disengagement from a caregiver indicate a

developing ability to control, or regulate, arousal (e.g., Fogel, 1993; Stifter & Moyer, 1991; Tronick, 1989). As infants learn to integrate affect with visual engagement, researchers observe an attenuation of negative affect and emotion lability from 3 to 6 months of age (Maltesta & Haviland, 1982).

Thus, from the first months of life, typically developing infants appear to learn “display rules” for facial expressions of emotion (Ekman & Friesen, 1975; Izard, 1971, 1977; Sroufe, 1979; Tomkins, 1962, 1963). The ability to engage in interaction (i.e., directing smiles) while also regulating arousal (i.e., disengaging attention) is thought of as a developmental precursor to more complex referential communication (e.g., joint attention; Messinger & Fogel, 2007). The infant appears to develop these foundational abilities through experience in interaction contexts that vary, from moderately arousing (i.e., face-to-face interaction) to highly arousing (i.e., peek-a-boo; Fernald & O’Neill, 1993; Sroufe & Waters, 1976).

These early interaction experiences appear to shape the development of facial expression processing. Infants are born with some capacity to process facial expressions (Nelson, 2001). In fact, they discriminate facial expressions as early as 36 hours after birth (Field, Woodson, Greenberg, & Cohen, 1983). In addition, infant’s activity in contexts where social signals of emotion are prevalent provide a context for learning about the perceptual qualities of facial expressions (Leppanen & Nelson, 2009; McClure, 2000). In fact, tuning the perceptual systems to complex parameters of facial expressions occurs across the first years of life. This process provides evidence for the experience, or activity, dependent nature of emotion development.

The progressive neurocognitive discrimination and recognition of happy faces during the first year of life is thought to be the result of experience with smiles during mother-infant interaction (e.g., Ludemann & Nelson, 1988). Typical infants discriminate happy faces by 3



months of age, before they are able to discriminate other types of facial expressions (e.g., Barrera & Maurer, 1981; Young-Browne, Rosenfeld, & Horowitz, 1977). At 4 months infants look longer at smiling faces than angry, sad, or neutral faces (LaBarbera, Izard, Vietze, & Parisi, 1976; Oster & Ewy, 1980). By 5 to 7 months, infants' attentional biases indicate an ability to categorize smiling facial expressions (e.g., Bornstein & Arterberry, 2003; Ludemann & Nelson, 1988). By 7 months, typically developing infants' electrophysiological activity shows neural-level discrimination of happy expressions from other types of facial expressions (Nelson & de Haan, 1996). This perceptual tuning is observed first for facial expressions of happiness, with perceptual tuning to other types of emotional expression emerging later in the development when the changes in the infant's activity (e.g., the development of crawling) make other facial expressions (e.g., fear) more salient (Nelson, Parker, Guthrie, & The Bucharest Early Intervention Project Core Group, 2006).

Disruption of the neural and perceptual systems of an infant to process social signals of emotion, disruption of infants' emotional experience in an interaction context, or disruption of both processes could contribute to atypicalities in emotion perception and production that partly characterize the ASD phenotype. Infant sibling studies provide a method for studying this potential disruption. Infant sibling studies of emotion perception and production published to date are reviewed below.

## **1.5 FACIAL EXPRESSION PRODUCTION AND PERCEPTION IN INFANTS AT HR FOR ASD**

Infant sibling researchers have assessed aspects of emotional development as a marker of HR status (e.g., Cassel et al., 2007; Merin, Young, Ozonoff, & Rogers, 2007; Yirmiya et al., 2006)

and predictor of ASD (Ozonoff et al., 2010; Rozga et al., 2011; Young, Merin, Rogers, & Ozonoff, 2009). The majority of these studies have assessed observable behavior, focusing on the production of facial expressions in the context of the face-to-face interaction. Only two published studies have assessed facial expression perception in an infant sibling sample (Merin et al., 2007; Young et al., 2009). Infant sibling studies examining facial expression production will be reviewed first, followed by studies of facial expression perception.

Thus far, all studies assessing HR and LR differences in facial expression production in an infant sibling sample have utilized the Face-to-Face Still Face (FFSF) paradigm (Tronick, Als, & Brazelton, 1977). In this interaction paradigm parents first engage in responsive play with their infants, are then asked to be non-responsive (i.e., maintain a “still face”), and finally resume responsive play. For typically developing infants, decreased parent responsiveness elicits the “still face effect”: decreased parent-directed gaze, positive affect, and increased negative affect (e.g., Tronick, Adamson, Wise, & Brazelton, 1978). Infant sibling researchers have predicted that the shifts in interactive context which define this paradigm may elicit different gaze and affective response from HR versus LR infants.

Despite using a similar methodology (i.e., the FFSF paradigm) across investigations, infant sibling studies exploring risk group differences in expression production present mixed findings. As a whole, significant results indicate a more muted affective profile in HR compared to LR infants during the first year of life. HR infants display more neutral affect at 4 months (Yirmiya et al., 2006) and less smiling at 6 months (Cassel et al., 2007) than their LR counterparts. Still, some studies report no risk group differences in expressed affect (e.g., Merin et al., 2007). Conflicting findings from a limited number of studies make it difficult to draw summative conclusions about facial expression production as a marker of HR for ASD.

Surprisingly, the most consistently reported finding is *similarity* between HR and LR infants' affective response to the still phase episode (Cassel et al., 2007; Merin et al., 2007; Yirmiya et al., 2006), with risk group differences observed most consistently in normative interaction periods of the FFSF paradigm. These data have led some researchers to suggest that HR and LR infants' affect may appear most dissimilar in the context of *normative* face-to-face play (Cassel et al., 2007). Follow-up investigations, indicating whether these early risk-group differences marked HR status only or predicted later ASD diagnosis in these infant sibling cohorts, have not been published.

To date, only two infant sibling studies have assessed facial expression production as an early predictor of ASD diagnosis (Ozonoff et al., 2010; Rozga et al., 2011). Both investigations measured other-directed looking and smiling in the context of face-to-face interaction at 6 months and later age points. In both studies, decreased directed looking and smiling at and after 12 months of age predicted to ASD diagnosis in toddlerhood. Neither group reported significant differences between affected and unaffected individuals looking and smiling during the first year of life. Still, it is important to note that both studies found a non-significant trend for 6-month-olds later diagnosed with ASD to display *more* social engagement (e.g., more frequent social smiles) than those found to be unaffected. Thus, if these behaviors do contribute to the early autism phenotype prior to 12 months of age, it is possible that *increased* positive arousal is indicative of emerging ASD.

Similar to infant sibling studies investigating facial emotion production, the literature documenting infant siblings' facial affect perception is limited. In fact, looking to facial expressions has been assessed in only one infant sibling cohort (Merin et al., 2007; Young et al., 2009). Merin and colleagues (2007) utilized eye-tracking to assess HR and LR infants' looking

to their mother's face during the FFSF paradigm. Looking behavior was not shown to distinguish HR from LR infants during the naturalistic portions of the paradigm, when mothers augment their facial expressions. However, a subgroup of 11 infants, 10 of whom were HR, demonstrated diminished gaze to their mother's eyes relative to mouth during the still-face period (i.e., when mother's faces are held in a neutral expression). Thus, looking to faces, not faces that varied by expression, appeared to mark HR for a subset of infants. In a follow-up study, which followed these infants to diagnostic outcome, this looking pattern was not shown to predict ASD diagnosis (Young et al., 2009).

In sum, infant sibling studies of emotion production and perception are limited in number, which makes overall conclusion about emotion development difficult to formulate. The largest subset of the infant sibling studies that have assessed emotion development have focused on emotion production as a characteristic that may distinguish HR and LR infants. Findings from these studies are mixed. Therefore, the qualities of affect production that define HR status remain unclear. In addition, studies following these HR cohorts to diagnostic outcome have not published. Therefore, it is not clear whether reported risk group differences in expressed affect are markers of HR or are driven by infants in the cohort who go on to develop ASD. The two studies that have assessed affect production as a predictor of ASD find that decreased directed looking and smiling predict ASD at 12 months, find no significant predictors of ASD prior to 12 months, but note a non-significant trend for increased directed smiling among 6-month-olds later diagnosed with ASD (Ozonoff et al., 2010; Rozga et al., 2011). Finally, the only published infant sibling investigation of emotion perception indicated that a subset of HR and LR infants looked at faces in a manner typical of individuals with an ASD diagnosis (i.e., diminished looking to the eyes relative to the mouth; Klin et al., 2002), but follow-up studies showed that

this visual attention pattern did not predict to ASD diagnosis (Young et al., 2009). Altogether, infant sibling studies that investigate emotion development are few and indicate the need for further research.

## **1.6 APPLICATION OF EXPERIENCE-EXPECTANT & TRANSACTIONAL MODELS TO INFANT SIBLING STUDIES**

To date, infant sibling investigations have been driven by the assumption that what defines ASD in toddlerhood and beyond will likely define HR and ASD in infancy. Because a diagnosis of ASD from toddlerhood onward is based on behavioral observation of social communication, social reciprocity and the presence of restricted/repetitive behaviors (American Psychiatric Association, 2000, 2013), infant sibling researchers have looked for behavioral markers in these areas first. Behavior in these domains has been shown to reliably predict ASD at or after 12 months of age (e.g., Ozonoff et al., 2010; Rogers, 2009), but the qualities that appear to predict ASD prior to 12 months of age are anomalies of brain structure, function, and aspects of visual perception (e.g., Bosl, Tierney, Tager-Flusberg & Nelson, 2011; Chawarska, Macari, & Shic, 2013; Wolff et al., 2012). Findings indicating a later than expected onset of behavioral symptoms and the early emergence of neurocognitive predictors have led the research community to search for a theoretical model that explains this pattern of development. A number of researchers have suggested impairments in experience-expectant (i.e., neurological and perceptual) mechanisms having cascading downstream effect on behavior that lead to the emergence of social disability in the second year of life (e.g., Chawarska et al., 2013; Hutman, 2013; Jones & Klin, 2013).

Experience-expectant theories help explain emotion-specific findings reported in infant sibling studies and guide future investigations. Experience-expectant theories of typically developing infants' emotion development postulate that neurological and perceptual systems “expect” emotion-specific activity and experience. It is possible that HR infants, and/or infants who go on to develop ASD, come into this world with atypicalities in the neurocognitive systems that process emotion-laden cues. Over time, even slight differences in these experience-expectant mechanisms (i.e., neurological responses or visual attention) could have cascading effects on HR infants' ability to produce facial expressions in a typical manner, and may contribute to disruption of arousal regulation.

However, it is also possible that there are differences in the emotion-specific activity (i.e., behavior) of HR infants and/or infants who go on to develop ASD. The typical infant literature, and transactional models of development (Sameroff, 1975, 2009), suggests that neither system, neurocognitive or behavioral, works in isolation. The systems for emotion processing and production develop in tandem, affecting one another across time. Thus, studying both emotion production and perception in the same sample is paramount to understanding the emergence of these abilities in an infant sibling sample.

Considering this infant siblings' emotion development from a transactional perspective, it is also important to assess the influence of the contextual factors. The neurocognitive and behavioral abilities of the infant to produce and perceive emotional social signals are influenced and augmented by feedback from the external context. The behavior of an interaction partner in a face-to-face interaction, the type of interaction context (i.e., a highly arousing context like peek-a-boo, or moderately arousing context like face-to-face interaction), and the intensity of facial expressions of emotion displayed in the environment are factors that shape the perception

and/or production of emotion in typical infancy. These factors should be assessed as potentially influencing emotion development in an infant sibling sample.

To explore the complex interplay among emotion perception and emotion production that may contribute to the unfolding of ASD, infant sibling studies should simultaneously investigate development in both domains of development. Leppanen and Nelson's (2009) experience-expectant theory of typical emotion development provides a theoretical rationale for this type of investigation. Considering Sameroff's (1975, 2009) transactional theory of social development, infant sibling studies of emotion should also consider the interplay between the external social-emotional context and the emotion processing and production abilities of the individual. These contextual variables influence the emergence of social-emotional abilities from infancy. Thus, the current study was designed to consider the quality of and interplay between emotion perception and production abilities across the first year of life in infant siblings, as well as features of the external context that may influence this development.

## **1.7 THE PRESENT STUDY**

The present study consisted of two procedures; one designed to examine *emotion production* and the other *emotion perception* of infants at HR for ASD during the first year of life. *Emotion production* was defined as infants' facial expressions and associated looking behavior produced in the context of mother-infant interaction. *Emotion perception* was defined as visual attention to still images of emotion-laden faces measured in an eye-tracking paradigm. Aims and hypotheses specific to each procedure are described below. This is followed by a more general

discussion regarding the possible integration of findings from emotion production and perception tasks.

### **1.7.1 Emotion Production**

The current study's emotion production paradigm focused on 6- and 11-month-old infant siblings' production of facial expressions and looking behaviors in the context of typical mother-infant face-to-face interaction. Infant sibling researchers have been unable to clearly determine whether the nature and emergence of facial expression production abilities may serve as an early marker of HR and/or ASD diagnosis (e.g., Cassel et al., 2007; Ozonoff et al., 2010; Rozga et al., 2011; Yirmiya et al., 2006). Thus, the main goal of the emotion production task was to further explore the emotional experience of infant siblings during the first year of life. Specific aims regarding infant behavior were to: a) assess group differences in HR and LR infants' use of gaze and facial expressions during face-to-face interaction at 6 and 11 months of age and b) explore whether gaze and affective behavior at 6 and/or 11 months of age predicts diagnostic outcome in toddlerhood. A secondary aim was to explore and define mother's social signals of emotion. Although it is widely acknowledged that mothers are an influential partner in the affective interactions of typically developing infants (e.g., Beebe & Lachmann, 1988), infant sibling studies have described the infant's affect, but not mother's affective behavior. The current study assessed mothers' production of facial expressions in order to determine a) if mother's behavior is influenced by knowledge of their infants' risk-status, and b) how findings about mother's behavior may or may not augment conclusions made about infants' behavior.

**Predictions.** Four sets of predictions were made regarding the facial expression production of infant siblings and their mothers. The first set of predictions suggests how infants'



emotion production may characterize HR status, while the second set suggests how emotion production may characterize those infants later diagnosed with ASD. A third group of hypotheses suggests how interaction context may assist in the differentiation of HR from LR infants and the characterization of those infants later diagnosed with ASD. The final set of predictions relate to mother's affect production. All predictions are described below.

Published infant sibling studies provide a foundation for hypotheses regarding risk group differences in emotion production. Prior studies have found that, when observed during normative face-to-face interaction, social signals of emotion distinguish HR from LR infants as early as 4 to 6 months of age (Cassel et al., 2007; Yirmiya et al., 2006). Therefore, it was hypothesized that social signals of emotion measured in the current study would also distinguish HR from LR infants at 6 months. It was further predicted that the nature of differences in social signals would be consistent with past reports, which found HR 6-month-olds show diminished gaze, smiling, and increased neutral expressions when compared to their LR age-mates (e.g., Cassel et al., 2007). A final hypothesis regarding risk-group differences was that these behaviors would no longer distinguish HR and LR groups at 11 months, as mother-infant face-to-face interaction typically becomes less salient over the course of the first year of life (Crawley, Rogers, Friedman, Iacobbo, Criticos, et al., 1978).

Hypotheses regarding the utility of social signals of emotion measured at 6 and 11 months to predict diagnostic classification in toddlerhood are also supported by prior infant sibling research. First, it was predicted that the behaviors that distinguished risk groups at 6 and 11 months may predict atypical development in toddlerhood. That is, it was hypothesized that these behaviors may predict general atypical development, which includes any classification in toddlerhood other than typical development (i.e., language delay, global developmental delay,

social concerns, and ASD). Second, consistent with findings of Ozonoff et al. (2010) and Rozga et al. (2011), it was predicted that decreased smiling, directed smiling, and other-directed gaze at 11 months would be specific predictors of ASD diagnosis. Third, because Ozonoff et al. (2010) and Rozga et al. (2011) also reported a non-significant trend for increased positive affect and/or other-directed looking at 6 months to predict ASD diagnosis (Ozonoff et al., 2010; Rozga et al., 2011), it was predicted that increased expression of these behaviors at 6 months may be significant predictors of ASD. The second and third hypotheses lead to a final, overarching prediction regarding the timing of emergence of emotion-specific behaviors as predictors of ASD. It was proposed that increased positive affect and other-directed looking may be predictive of ASD at 6 months, while diminished positive affect and other-directed looking may be predictive at 11 months of age.

A third set of predictions relate to the face-to-face interaction contexts of the current study (i.e., face-to-face interaction vs. peek-a-boo). It was hypothesized that change in face-to-face interaction context would augment the emotion production of HR and LR infants, such that risk-group difference would be more exaggerated in one context than another. Since it was predicted that HR 6-month-olds would display a more muted affective profile than their LR counterparts, it was also hypothesized that group differences would be most obvious in an interaction context that typically produces high levels of positive affect (i.e., peek-a-boo; Sroufe & Waters, 1976; Washburn, 1929). It was suspected that the interaction contexts of the current study (e.g., peek-a-boo) would “pull” for greater amounts of positive affect and other-directed smiling than the interaction contexts used in past studies (i.e., the FFSF paradigm and standardized cognitive assessments).

Finally, specific predictions were made about mothers' emotion production. It was predicted that infants' risk-status would not impact mothers' affective behavior. Still, it is important to clarify whether or not this is the case and published infant sibling studies have yet to assess mother's behavior. Significant differences in mother's behavior based on the risk of their infant would change the environment in which the infant was learning about emotions, and alter their experience. Such differences could have cascading effects on the tuning of neurological, perceptual and behavioral systems to signals of emotion.

### **1.7.2 Emotion Perception**

The current study's emotion perception procedure collected information about 6- and 11-month old infant siblings' visual attention to pairing of smiling and neutral faces in the context of an infant looking paradigm and utilizing eye-tracking methods. No prior infant sibling studies have utilized both of these methods to study infant siblings' visual attention to social signals of emotion, although they are common to studies of typically developing infants' perception of emotion (e.g., Amso, Fitzgerald, Davidow, Gilhooly, & Tottenham, 2010; Gredeback, Johnson, & von Hofsten, 2009; Peltola, Leppanen, Vogel-Farley, Hietanen, & Nelson, 2009). This procedure was used with the primary aim of assessing looking behavior as a potential marker of HR and/or ASD. Specific aims were to: a) assess group differences in HR and LR infants' looking to neutral and smiling facial expressions at 6 and 11 months of age and b) explore whether visual attention to these stimuli, measured in an eye-tracking setting, predict diagnostic classification in toddlerhood. Specific predictions were made regarding risk group differences and the predictive utility of *visual preference* and *visual exploration*.

**Predictions.** Two predictions regarding *visual preference* were made. First, it was predicted that LR 6- and 11-month-olds, but not HR 6- and 11-month-olds, would demonstrate a visual preference for smiling over neutral faces. Furthermore, it was predicted that this visual preference would predict diagnostic classification in toddlerhood. Second, it was predicted that preference for smiling over neutral faces for HR and LR infants at 6- and 11-months would vary based on the intensity of the smiling face displayed (i.e., low-level to exaggerated smile). It was predicted that there would be greater differences between HR and LR infants for the extreme ends of the smile intensity spectrum. In other words, HR and LR infants' visual preference would differ to a greater degree when smile-neutral pairs included a low-level smile or exaggerated smile, than when smile-neutral pairings included a prototypical smile. It was further predicted that this visual preference, varying by smile intensity, would predict diagnostic outcome.

Two predictions regarding *visual exploration* were made. These predictions were based on findings from studies of the face processing abilities of older individuals with an ASD diagnosis. Research has shown that individuals with ASD visually attend to facial expressions in an atypical manner (e.g., Pelphrey et al., 2002). This atypicality is displayed in older individuals with ASD, suggesting that atypical development of facial expression perception may be present from infancy. Studies of individuals with an ASD diagnosis indicate increased looking “off the face” than “on face” (e.g., Klin et al., 2002; Chawarska, Macari, & Shic, 2012), more looking to the outer features of the face (i.e., hair, forehead, cheek area) than the internal features of the face (i.e., eyes, nose, and mouth; Chawarska & Shic, 2009; Pelphrey et al., 2002), and more looking to the mouth than eye region of a face (e.g., Boraston, Corden, Miles, Skuse, & Blakemore, 2008; Chawarska & Shic, 2009; Dalton et al. 2005; Pelphrey et al. 2002) in individuals with

ASD as compared to typically developing and developmentally delayed controls. Based on these findings, the current study predicted risk group differences in visual exploration to these areas at 6 and 11 months of age. In addition, it was hypothesized that risk group differences in visual exploration of these areas would predict diagnostic classification in toddlerhood.

### **1.7.3 Overarching Goals**

An overarching goal of the present study was to integrate findings across emotion production and perception tasks in order to make concluding statements about the relations between and timing of developments in emotion-specific perceptual and behavioral systems. The nature of emotion production and perception that unfolds in an infant sibling cohort during the first year of life, and the timing of their emergence as markers of risk and predictors of ASD diagnosis, could inform the field about the complex unfolding of ASD during the first year of life.

## **2.0 METHODS**

### **2.1 PARTICIPANTS**

Two groups of mother-infant dyads participated in the current study. The groups of mother-infant dyads were distinguished by the infants' risk for ASD diagnosis. Infants were either the infant siblings of a) children with an ASD diagnosis (HR infants) or b) typically developing children (LR infants). Infants entered the study at 6 or 11 months of age. Part of a larger, longitudinal infant sibling study conducted through the University of Pittsburgh's Autism Center of Excellence (ACE), participants were recruited within western Pennsylvania via professional referrals, referrals through the Autism Research Program at the University of Pittsburgh, local agencies and schools serving individuals with ASD, parent support organizations, and advertisement in local magazines and television programming.

General criteria for infants' inclusion in the current study were English as the primary language spoken in the home, singleton birth status, and birth at or after 34 weeks gestation. Infants were excluded if there was a history of pregnancy or birth complications, low birth weight (i.e., below 4 lbs.), traumatic brain injury, severe birth defects, or known genetic syndrome. Additional inclusion criteria for HR infants was having at least one older sibling with a diagnosis of ASD, which was confirmed using gold-standard diagnostic instruments, including the *Autism Diagnostic Observation Schedule-Generic* (ADOS-G, Lord et al., 2000) or *Autism*

*Diagnostic Interview-Revised* (ADI-R; Lord, Rutter, & LeCouteur, 1994), and medical record review. Additional inclusion criteria for LR infants was being the later-born sibling of one or more typically developing children without the presence of an ASD diagnosis in the immediate family, first, or second degree relatives.

Infants included in this investigation completed study procedures at 6 months, 11 months, or at both age points. A total of 107 mother-infant dyads participated in a face-to-face interaction paradigm assessing production of social emotional cues (i.e., infant smiling and looking behavior, and mother's smiling) and a total of 128 infants participated in an eye-tracking procedure assessing emotion perception (i.e., infants visual attention to smiling and neutral faces). At 6 months, 50 mother-infant dyads (LR: 24, HR: 26) participated in the face-to-face interaction paradigm and 59 infants (LR: 28, HR: 31) participated in the eye-tracking procedure. At 11 months, 57 mother-infant dyads (LR: 33, HR: 24) participated in the face-to-face interaction paradigm and 69 infants (LR: 32, HR: 37) participated in the eye-tracking procedure. At both age points, HR and LR groups did not differ by gender distribution, age, verbal or nonverbal abilities. Demographic information for infants and mothers who participated in study procedures at 6 and 11 months is presented in Tables 1 to 4.

Information regarding participant overlap across emotion production and perception procedures is presented in Table 5. A total of 42 6-month-olds (LR: 23, HR: 19) and 53 11-month-olds (LR: 30, HR: 23) participated in both paradigms. Some infants participated in the interaction sequence, but not the eye-tracking paradigm: 1 LR 6-month-old, 7 HR 6-month-olds, 3 LR 11-month-olds, and 1 HR 11-month-old. Others participated in the eye-tracking paradigm, but not the interaction sequence: 5 LR 6-month-olds, 12 HR 6-month-olds, 2 LR 11-month-olds, and 14 HR 11-month-olds.

There are general reasons for missing data, including: a) infant fussiness or fatigue, b) equipment malfunction, and c) experimenter error. There were also trends apparent in the missing data. For instance, it was more common for interaction sequence data to be missing than eye-tracking data. It was also more common for HR infants with eye-tracking data to have missing interaction sequence data than it was for LR infants with eye-tracking data to have missing interaction data.

There are three reasons why interaction sequence data were missing more often than eye tracking data. The primary reason is that collection of eye-tracking data was prioritized over the collection of interaction sequence data because eye-tracking studies were being conducted as part of the ACE project, while the interaction paradigm was not part of this larger project. The second reason is that eye-tracking data collection was initiated before interaction procedure data collection. The final reason that interaction data was missing more frequently than eye-tracking data is that the interaction procedure required infants' mothers to participate in the paradigm. Therefore, if an infant's father brought them to the study appointment, the interaction paradigm was not completed.

There are also two reasons why HR infants with eye-tracking data have more missing interaction paradigm data than LR infants. The primary reason is that HR infants were harder to locate and recruit as participants, which made their time more valuable and made it more likely that they would only participate in ACE project procedures (i.e., eye-tracking). The second reason for this trend is that there were periods of time when there was an influx in HR infant enrollment and eye-tracking paradigm data were being collected, but interaction paradigm data were not.



A subset of HR and LR infants that participated in study procedures at 6- and/or 11-months was seen for at least one diagnostic outcome visit at 24-, 36-, and/or 48-months of age. Thus, diagnostic outcome could be determined for a subset of 6- and 11-month-olds who participated in the face-to-face interaction paradigm: 20 of 24 LR 6-month-olds (83%), 24 of 26 HR 6-month-olds (92%), 26 of 33 LR 11-month-olds (79%), and 23 of 24 HR 11-month-olds (96%). Diagnostic outcome was also determined for a proportion of the infants who participated in the eye-tracking procedure: 21 of 28 LR 6-month-olds (75%), 20 of 31 HR 6-month-olds (65%), 26 of 32 LR 11-month-olds (81%), and 35 of 37 HR 11-month-olds (95%). In all but three cases, diagnostic classification could not be determined during the study period because infant participants had not yet reached diagnostic outcome age points. For three participants (LR: 2, HR: 1) diagnostic outcome could not be determined because the family dropped out of the study before the infant reached diagnostic outcome. Diagnostic classification data for the 6- and 11-month-olds are summarized in Tables 6 to 9. A description of how classifications were made is presented below, under *Diagnostic Classification*, and diagnostic classification criteria are presented in Table 10.

## **2.2 PROCEDURE**

Informed consent was obtained from parents upon infants' enrollment. Mother-infant dyads were observed in a laboratory setting. At 6 and 11 months, mother-infant dyads participated in a face-to-face interaction sequence, infants participated in an eye-tracking paradigm assessing visual attention to neutral/smile face pairs, and infants' cognitive functioning was assessed using

a standard measure. A subset of infants was seen for follow-up diagnostic outcome visits at 24, 36, and/or 46 months. A detailed description of measures completed at infant time points and diagnostic outcome time points is provided below.

### **2.2.1 Measures Administered at 6 and 11 Months**

At 6 and 11 month age points, infants participated in the face-to-face interaction paradigm aimed at assessing emotion production, an eye-tracking paradigm aimed at assessing visual attention to facial expressions of emotion, and a standardized assessment of cognitive functioning.

***Face-to-face interaction paradigm.*** Mother-infant dyads participated in identical, two-part face-to-face interaction sequences at the 6- and 11-month visits. The interaction sequence was three minutes in duration. The first two minutes of the interaction were defined as face-to-face interaction (FTF). Directly following this, mothers engaged in one minute of peek-a-boo (PAB; see Table 11 for mean duration of FTF and PAB episodes for HR and LR 6- and 11-month-olds). Prior to beginning the interaction paradigm, mothers were given detailed instructions. They were asked to engage with their infants as they “normally would” during FTF. They were asked to play PAB in a standard way: by cover their face, saying “Where’s [infants’ name]?”, and then uncovering their face saying “Peek-a-boo” or “There he/she is.” They were asked to refrain from touching their infant during the interaction sequence and from introducing objects, such as toys, during their interaction. Cue cards indicating what to do first (i.e., “interact as you normally do”) and second (i.e., “play peek-a-boo”) were hung on a bulletin board on a wall within the mother’s view. Mothers were told that they would hear a knock on a one way mirror that would indicate to them when they should move from FTF to PAB. The

interaction began only after mothers acknowledge that they understood these instructions and infants were comfortably seated in a high-chair across a small table from their mothers.

*Apparatus.* Two cameras, placed in the corners of the room where the interaction took place, recorded the interaction sequence. One camera captured the mothers' upper body and face, while a second camera captured the infants' upper body and face. A microphone, placed in the room, recorded the auditory component of the interaction. Video of the mother and infant were recorded on a split screen, with video and audio data being integrated by a Panasonic Digital AV Mixer, DJ-MX30. Audiovisual data were recorded onto a DVD using a Panasonic DVD recorder, model DMR-EZ27.

*Infant-specific coding and reliability.* All facial affect and looking behavior displayed by the infant during the three minute mother-infant interaction was coded. Infants' looking behavior and facial affect was coded in separate passes by three coders blind to infant risk-status. The Facial Action Coding System (FACS; Ekman & Friesen, 1978) and its application to infants, BabyFACS (Oster, 2009; Unpublished monograph and coding manual), was utilized to code infants' facial affect. Coders were certified in FACS and trained in BabyFACS. Infants smiling, negative affect, and neutral affect were coded. Smiling was defined by the upward turning of the lip corners (AU 12). Negative affect was defined by display of a cry-face, the lateral stretching of the lips (AU20) and lowering of the brow (AU4). Neutral affect was defined as any facial display not categorized as smiling or a cry-face. Infant looking codes included a) looking at mother (face or body) and b) looking away from mother. Directed smiling was defined as time infants spent both "looking at mother's body" and smiling. See Table 12 for more detailed operational definitions of infant facial affect and looking codes.

Exactly 14% of FTF and PAB interaction videos at the 6-month age point and 12% of FTF and PAB interaction videos at the 11-month age point were coded for inter-rater reliability. The mean percent agreement between the primary coder and two additional coders was calculated for FACS codes (i.e., smiling, negative and neutral affect) and looking variables separately. Cohen's kappa (Cohen, 1960) was also calculated to assess agreement between the primary coder and both additional coders, with  $k \geq 0.70$  (e.g., Cassel et al., 2007) considered acceptable inter-rater reliability for all variables. Mean percent agreement between the primary coder and additional coders for FACS codes ranged from 76-84% across interaction contexts and infant age points. Cohen's kappa values assessing agreement between the primary and additional coders were  $\geq 0.71$  for FACS codes across interaction contexts and infant age points. Mean percent agreement between the primary coder and additional coders for infant looking codes ranged from 83-90% across interaction contexts and infant age points. Cohen's kappa values accessing agreement between the primary and additional coders were  $> 0.70$  for infant looking codes across interaction contexts and infant age points. See Table 13 for more detail regarding reliability, including mean percent agreement and Cohen's kappa values for primary coder and each additional coder by interaction context and infant age point.

*Mother-specific coding and reliability.* Mother's affect and affective intensity was measured using a global rating scale. The rating scale ranged from 0 to 3. A 0 indicated that the mother's face could not be seen (i.e., out of camera view or behind her hands), a 1 indicated neutral affect, a 2 indicated mild to moderate positive affect, and a 3 indicated high positive affect. These codes were assigned based on facial expression only and codes were based on the FACS codes typically used to code adult smiles (Ekman & Friesen, 1978). Detailed descriptions of each of these codes can be found in Table 14. A global rating of 0 to 3 was given every 5

seconds during the interaction paradigm. These global ratings for each 5 second block were then averaged (disregarding ratings of 0) to calculate an overall global rating from mother's affect during the entire FTF and PAB segments.

Reliability was established between two raters, blind to the risk status of the infant-mother dyad. For the 6-month time point, 18% of the FTF and PAB interaction segments were randomly selected to be coded for inter-rater reliability. For the 11-month time point, 19% of the FTF and PAB interaction segments were randomly selected and coded for inter-rater reliability. At 6-months, mean percent agreement was 86.62 for FTF and 76.83 for PAB. At 11-months, mean percent agreement was 90.12 for FTF and 88.57 for PAB. These calculations reflect the average percent agreement between the ratings of the two coders at the 5-second interval level. Pearson's product-moment correlation coefficients were calculated to assess inter-rater reliability at the level of overall ratings. Thus, these correlations reflect agreement in the global rating assigned to mother's affective behavior, which was the average of ratings given every five seconds during an interaction. One overall rating score was given for each interaction context (i.e., FTF and PAB). The results of these analyses indicated an acceptable level of inter-rater reliability. At the 6 month time point, agreement ranged from  $r = 0.83$  for FTF to  $r = 0.74$  for PAB. At the 11 month time point, agreement for FTF and PAB was  $r = 0.89$ . Reliability meetings occurred regularly to prevent coder drift following establishment of initial reliability. Coding disagreements were resolved through consensus coding.

***Emotion perception eye-tracking paradigm.*** To assess visual attention to facial expressions of affect, 6- and 11-month-olds were placed in an infant high chair facing a projection movie screen (69 X 91 cm) and Tobii X120 stand-alone eye-tracker (see Figure 1). Each infant sat approximately 162 cm from a projection screen and 81 cm in front of Tobii X120

eye-tracker. The testing area was surrounded by black curtains to reduce distractions. Stimuli were rear projected onto the screen using Tobii Studio software, and eye movements were recorded by a Tobii X120 stand-alone eye tracker at a sampling rate of 60 Hz, accuracy of 0.5 degrees of visual angle, spatial resolution of 0.2 degrees, and drift of 0.3 degrees. A Dell Dimension 9200 displayed experimental stimuli and recorded eye-movement data. Eye-tracking data were processed using Tobii Studio software, Version 2.0.6.

*Stimuli.* Pairings of static smiling and neutral female faces were projected onto the screen using a visual paired comparison procedure (Fantz, 1956). Stimuli were taken from a standardized set of static facial expression images called the NimStim Face Stimulus Set. Faces chosen were Caucasian and female. Research has shown high agreement between children and adults regarding the facial expressions displayed by this standard set of face stimuli (Tottenham, Borscheid, Ellertsen, Marcus, & Nelson, 2002). As part of the visual paired comparison procedure, infants were presented with three levels of face pairings with each level distinguished by variation in smile intensity: neutral/closed-mouth smile (low intensity), neutral/open-mouth smile (moderate intensity), and neutral/exaggerated smile (high intensity; see Figure 2). Within a pairing the women's identity was held constant, but identity varied between pairing presentations. Each infant was shown all three intensity levels of neutral/smile pairings. There were four trials per level, two different identities per level, with one identity pairing followed directly by its' left/right reversal. Therefore, in total each infant saw three levels of intensity pairings over 12 total experimental trials. Between presentations of each pairing, an attention grabbing animation was displayed at the center of the screen to regain the infants' looking and focus visual attention at the center of the screen before presentation of the next face pair (see Figure 3). Each neutral/smile face pair was presented for a total of four seconds.

*Procedure.* While infants were being positioned in the high chair an infant-friendly video (i.e., Baby Mozart) was rear-projected onto the screen. Caregivers sat next to their infant for the duration of the eye-tracking paradigm in order to reassure and soothe their infant when necessary. Caregivers were asked to encourage their child to look at the screen during testing, but were asked not to label images that were displayed. Once the infant was seated in the high chair, the chair was moved forward, backward, right and left until the infants' gaze was detected by the eye-tracker. The calibration procedure (see *Calibration Procedure* description below) was then initiated. When calibration was achieved, presentation of neutral-happy face pairings began using the visual paired comparison procedure described above.

*Calibration procedure.* The Tobii Studio software program allowed for calibration of the infants' gaze based on the infants' position from the eye-tracker. Once the experimenter manipulating the Tobii Studio program saw that the eye-tracker detected the gaze of the infant, as indicated on the computer screen, he/she initiated the infant calibration procedure. An animated toy rattle appeared in each corner and middle of a white screen (i.e., five calibration points). The animated rattle moved and emitted a sound at each point. The experimenter tracked the infants' eye movements to each calibration point, moving the calibration forward from point to point once the infant had fixated on each area. Following this calibration sequence, an infant-friendly video was switched on and the experimenter evaluated the calibration sequence. If the eye-tracker and Tobii Studio software detected both the right and left eye of the infant at each calibration point, the calibration was accepted and considered complete. If not, the calibration procedure was rerun until this was the case.

*Eye-tracking data exclusion.* Advances in corneal reflection technology have made eye-tracking methodology increasingly popular in the assessment of infant development (Gredeback,

Johnson, & von Hoftsten, 2009), a field that has long utilized infants' visual attention as a means of observing early developing interests and cognition (e.g., Fantz, 1956). With technological advances have come recommended guidelines for publishing eye-tracking data using an infant sample (Oakes, 2010). These guidelines include providing details about the eye-tracking system and calibration procedure (described above). Guidelines also recommend describing how eye-tracking data was excluded (e.g., if infants did not provide sufficient data on enough trials, trials on which infants failed to accumulate sufficient looking). For the current study, only two infants' eye-tracking data was excluded. Both infants were 6-month-olds at LR for ASD. These infants' eye-tracking data was excluded based on the following criteria: the infants displayed valid looking time data for only one stimulus pair.

*Areas of interest (AOIs).* AOIs were defined using Tobii Studio software in order to assess risk group differences in, and the diagnostic predictive utility of, the above noted looking variables (e.g., ratio of looking “on”/”on” and “off” the face). In eye-tracking studies AOIs define regions of stimuli where total looking time data are most relevant, considering the studies' specific research questions. For each neutral/smiling face pair the following AOIs were created (see Figure 4 for a depiction of AOIs): total stimulus area, right side of stimulus (smiling or neutral face depending on left/right reversal of stimulus), left side of stimulus (smiling or neutral face depending on left/right reversal of stimulus), total face area (i.e., all parts of the face for both smiling and neutral expression), total “off face” area (i.e., total stimulus area minus the total face area), inner features (i.e., eye + mouth + nose region), outer features (i.e., total face area minus the inner features), eye region, and mouth region.

*Standardized measures.* At 6 and 11 month age points the *Mullen Scales of Early Learning* (MSEL; Mullen, 1995) was administered to infants by research staff trained by a



licensed clinical psychologist. The MSEL is a standardized measure of general cognitive functioning that is administered to children 0-68 months and includes five subscales: Visual Reception, Fine Motor, Gross Motor, Receptive Language, and Expressive Language. Based on this assessment, verbal and nonverbal developmental quotients (DQ) were calculated (see Rogers, Hepburn, Stackhouse, & Wehner, 2003 for DQ calculation) to estimate language and cognitive functioning of 6- and 11-month-old HR and LR infants (see Tables 1 to 4).

## **2.3 DETERMINATION OF DIAGNOSTIC OUTCOME**

### **2.3.1 Standardized Assessment Measures**

Three standardized measures were used to assist in diagnostic classification at 24, 36, and 48 months of age. The MSEL (Mullen, 1995), a standardized measure of general cognitive functioning appropriate for children 0-68 months, was used to assist in determination of typical versus delayed cognitive development. The Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord et al., 2000), a structured play measure designed to elicit behaviors diagnostic of ASD and considered the “gold standard” in ASD diagnosis, was used to determine social concern and diagnose ASD. Importantly, the ADOS-G was administered by evaluator blind to infant risk status and each administration was conducted or reviewed by research reliable evaluators. Finally, the MacArthur-Bates Communication Development Inventory (CDI; Fenson et al., 2002), a parent report measure of children’s verbal and nonverbal communication, was administered at 24 months only in order to assist evaluators in determining diagnostic outcome, particularly language delays.

### 2.3.2 Determining Diagnostic Classification

Using the above mentioned standardized measures, HR and LR infants' diagnostic classification was determined at 24, 36, and/or 48 months. Diagnosis was conferred by consensus of licensed clinical psychologists experienced in the diagnostic assessment of young children with ASD and other developmental disabilities. Diagnosticians were blind to infants' risk status. Diagnostic classification categories were developed by clinical members of the University of Pittsburgh's ACE assessment core. These diagnostic categories included: *ASD*, *Language Delay without ASD*, *Global Developmental Delay without ASD*, *Social Concerns*, and *Typically Developing*. Diagnostic classification decisions were made based on the decision rules enumerated in Table 10.

Since each infant sibling had up to three opportunities to receive a diagnostic classification (24, 36, and 48 months), it was necessary to decide which diagnostic outcome time point would be utilized for analyses in the current study. The diagnostic classification utilized was the classification made at the infants' latest diagnostic outcome time point. For example, if the infant sibling was seen at 24-, 36-, and 48-months their 48-month classification was used. If the infant was seen at 24 months of age only, the 24-month diagnostic outcome was used. The diagnostic classification data for infants who participated in the study paradigms at 6- and 11-months are denoted in Tables 6 to 9.

## 2.4 ANALYTIC APPROACH

The analytic plan consisted of two separate sets of analyses for a) emotion production dependent variables derived from the face-to-face interaction paradigm and b) emotion perception dependent variables derived from the eye-tracking paradigm. Data analytic decisions were driven by the study's specific aims: to investigate emotion production and perception as a marker of risk *and* predictor of ASD diagnosis.

The first set of analyses examined emotion production and perception as *markers* of HR status. Repeated measures analyses of variance (ANOVA) were used to investigate the extent to which variables derived from the face-to-face interaction and eye-tracking paradigms differed between HR and LR infants at 6 and 11 months of age. At each age point, follow-up ANOVA were conducted to compare LR infants to the subgroup of HR infants with no known ASD diagnosis (HR-no ASD infants). This follow-up analysis was conducted to determine whether differences between the full HR and LR sample were the result of influence by the subset of HR infants who received a diagnosis of ASD at diagnostic outcome (HR-ASD infants). Because not all infants participated at 6 and 11 months of age, all analyses were performed cross-sectionally.

Findings from the above analyses provided the conceptual basis for the second set of analyses. These analyses assessed emotion production and perception variables as *predictors* of atypical development and ASD. Behaviors that were found to distinguish HR and LR infants were considered for inclusion as independent variables in a logistic regression equation predicting diagnosis. For variables derived from the face-to-face interaction paradigm, two different logistic regression analyses were performed at each infant age point (i.e., 6- and 11-months). The first regression type utilized the infants' affect and looking behaviors at 6 or 11

months to predict to any type of atypical development (i.e., global developmental delay, language delay, social concerns, or ASD). The second type of logistic regression used infants' affect and looking behavior at 6 or 11 months to predict an ASD diagnosis in toddlerhood. Similarly, for variables derived from the eye-tracking paradigm, two logistic regression analyses were performed at each age point. The first regression utilized visual attention within the context of the eye-tracking procedure to predict to any type of atypical development, while the second logistic regression used infants' looking behavior to predict an ASD diagnosis in toddlerhood.

## **3.0 RESULTS**

### **3.1 FACE-TO-FACE INTERACTION PARADIGM ASSESSING EMOTION PRODUCTION**

#### **3.1.1 Preliminary Analyses**

A preliminary examination of descriptive data for each dependent measure was conducted. Dependent measures included the proportion of interaction time spent smiling, crying, in directed smiling, in neutral affect, looking toward mother, and looking away from mother. The mean, standard deviations, and range of values for each variable are presented for HR and LR 6- and 11-month-olds in Tables 15 and 16, respectively.

An additional preliminary analysis investigated potential gender differences in infants' facial affect and looking. A multivariate ANOVA, including gender as a between-subjects variable, was conducted to assess whether male and female 6- and 11-month-olds differed on any dependent measure. No significant gender differences were found for any variable; therefore, infants' gender was not included as a covariate in subsequent analyses.

A final preliminary analysis assessed differences in maternal education level (MEL) by risk group. Two independent samples t-tests were conducted to determine if MEL differed for mothers of LR and HR infants at each infant age point. MEL was categorized as high school

education (1), some college (2), college degree (3), or graduate degree (4), with higher scores indicating higher educational achievement. There were significant differences in MEL by risk group at both 6 and 11 month age points. Mothers of LR 6-month-olds had a significantly higher level of education ( $M = 3.45$ ,  $SD = 0.71$ ) than mothers of HR 6-month-olds ( $M = 2.88$ ,  $SD = 1.08$ ),  $t(48) = -3.29$ ,  $p = 0.002$ . Mothers of LR 11-month-olds had a significantly higher level of education than mothers of HR 11-month-olds,  $t(55) = -2.3$ ,  $p = 0.03$ .

In follow up to these analyses, bivariate correlations between MEL and interaction paradigm infant-specific dependent variables (i.e., smiling, directed smiling, neutral affect, looking away, looking toward mother) were conducted. These correlations indicated that MEL was significantly correlated with infant smiling ( $r^2 = -0.29$ ,  $p = 0.04$ ) and neutral affect ( $r^2 = 0.29$ ,  $p = 0.04$ ) at 6 months during the FTF interaction, but not the PAB portion of the mother-infant interaction sequence. At 11 months, infant smiling ( $r^2 = -0.325$ ,  $p = 0.014$ ), directed smiling ( $r^2 = -0.390$ ,  $p = 0.003$ ) and crying ( $r^2 = 0.266$ ,  $p = 0.046$ ) were significantly correlated with maternal education, but only in the FTF context.

Still, MEL was not included as a covariate in subsequent ANOVA analyses of risk group difference in infant behavior for two reasons. First, visual examination of the data indicated that the correlation between MEL and dependent measures (e.g., smiling) are likely driven by the HR group only. There was a great degree of variability in MEL within the HR sample, but little variability in the LR sample (i.e., almost all mothers of LR infants had a high MEL). Analysis of covariance (ANCOVA) strongly requires that the variance of each group entered into analysis be similar. Second, there is a conceptual issue with covarying MEL in an assessment of potential risk group difference in infant behavior. Although MEL is known to be associated with maternal behavior, the effect on smiling in 6-month or 11 month old infants would logically only be

indirect via maternal behavior. In the current study, MEL was not significantly correlated with maternal behavior (FTF:  $r^2 = 0.008$ ,  $p = 0.94$ ; PAB:  $r^2 = -0.02$ ,  $p = 0.85$ ). For these reasons, MEL was not controlled for in subsequent analyses of infant behavior.

### **3.1.2 Mothers' Affect**

The next analysis assessed global ratings of mother's emotion production. This analysis was completed based on the premise that differences in mother's behavior due to infants' age, risk-status, or the interaction context, should be considered in the interpretation of infants' behavior. Thus, a 2 (risk-status) x 2 (age point) x 2 (interaction context type) repeated measures ANOVA was conducted, with risk-status and age as between-subjects variables and interaction context treated as a within-subjects variable. The analysis indicated that mothers displayed significantly more frequent high positive affect with their 6-month-old ( $M = 1.89$ ,  $SD = 0.23$ ) than their 11-month-old infants ( $M = 1.73$ ,  $SD = 0.03$ ),  $F(1, 104) = 10.19$ ,  $p < 0.001$ . In addition, mothers displayed significantly more high positive affect during PAB ( $M = 0.29$ ,  $SD = 0.03$ ) than FTF interaction ( $M = 0.27$ ,  $SD = 0.028$ ),  $F(1, 104) = 79.67$ ,  $p < 0.001$ . There were no significant main or interaction effects of risk-status, indicating that mother's facial affect displays did not differ based on their own knowledge of their infants' risk for ASD.

### **3.1.3 Infants' Facial Affect and Looking as Markers of HR**

Results of analyses investigating risk group differences in 6-month-olds' facial affect and looking are presented first, followed by results of similar analyses conducted with 11-month-

olds. At each age point, results of full-sample analyses are presented first, followed by presentation of findings from analyses that excluded HR-ASD infants. Only analyses of dependent measures that indicated a significant risk effect in the full-sample analysis were conducted for a second time with the exclusion of HR-ASD infants. A summary of ANOVA results conducted for the 6 and 11 month sample is presented in Tables 17 and 18, respectively.

***Full-sample analyses at 6-months.*** Separate 2 (risk-status) by 2 (interaction context) repeated measures ANOVAs were conducted to assess the impact of these independent variables on the proportion of interaction time infants spent engaged in smiling, directed smiling, crying, neutral affect, looking toward and away from their mother. Results are described for each dependent measure.

*Smiling.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA revealed a significant main effect of interaction context on the proportion of interaction time 6-month-olds spent smiling,  $F(1,48) = 27.07, p < 0.001$ . Across risk groups, 6-month-olds spent a significantly greater proportion of time smiling during PAB ( $M = 0.58, SD = 0.25$ ) than FTF ( $M = 0.41, SD = 0.27$ ). The analysis also indicated a significant interaction of risk-status and interaction context,  $F(1, 48) = 4.01, p = 0.051$ . LR 6-month-olds spent a significantly greater proportion of time smiling during PAB ( $M = 0.61, SD = 0.26$ ) than FTF ( $M = 0.36, SD = 0.26$ ),  $t(23) = -7.33, p < 0.001$ , while HR 6-month-olds smiling did not differ significantly across FTF ( $M = 0.46, SD = 0.28$ ) and PAB ( $M = 0.56, SD = 0.26$ ) contexts,  $t(25) = -1.714, p = 0.10$  (see Figure 5). HR 6-month-olds displayed a similar and high degree of smiling across FTF and PAB contexts.

*Directed smiling.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA also revealed a significant main effect of interaction context on the proportion of interaction time 6-month-olds spent in directed smiling,  $F(1, 48) = 64.03, p < 0.001$ . Infants directed their smiles



toward their mother for a significantly greater proportion of the time during PAB ( $M = 0.51$ ,  $SD = 0.25$ ) than FTF ( $M = .022$ ,  $SD = 0.18$ ). A significant interaction effect of risk and interaction context was also found,  $F(1, 48) = 5.54$ ,  $p = 0.02$ . In the context of FTF, HR 6-month-olds directed smiling to their mother for a greater proportion of time ( $M = 0.32$ ,  $SD = 0.22$ ) than LR 6-month-olds ( $M = 0.18$ ,  $SD = 0.16$ ),  $t(48) = 2.56$ ,  $p = 0.01$ . However, in the context of PAB, HR 6-month-olds ( $M = 0.49$ ,  $SD = 0.26$ ) and LR 6-month-olds ( $M = 0.50$ ,  $SD = 0.25$ ) spent a similar amount of interaction time in directed smiling,  $t(48) = -0.11$ ,  $p = 0.91$  (see Figure 6).

*Crying.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA revealed no significant main or interaction effects of these variables on the amount of crying displayed by 6-month-olds.

*Neutral affect.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context,  $F(1, 48) = 21.91$ ,  $p < 0.001$ . Overall, 6-month-olds spent a greater proportion of time in neutral affect during FTF ( $M = 0.55$ ,  $SD = 0.26$ ) than PAB ( $M = 0.41$ ,  $SD = 0.25$ ). The analysis also revealed a significant interaction of risk-status and interaction context,  $F(1, 48) = 7.36$ ,  $p = 0.01$ . LR 6-month-olds spent a significantly greater proportion of time in neutral affect during FTF ( $M = 0.62$ ,  $SD = 0.26$ ) than PAB ( $M = 0.39$ ,  $SD = 0.25$ ),  $t(23) = 6.19$ ,  $p < 0.001$ ; whereas HR 6-month-olds did not differ significantly in their display of neutral affect across FTF ( $M = 0.48$ ,  $SD = 0.24$ ) and PAB contexts ( $M = 0.42$ ,  $SD = 0.25$ ),  $t(25) = 1.17$ ,  $p = 0.25$  (see Figure 7).

*Looking toward mother.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA revealed a significant main effect of interaction context,  $F(1, 48) = 155.36$ ,  $p < 0.001$ . Across risk groups, 6-month-olds spent a greater proportion of time looking at their mother during PAB ( $M = 0.72$ ,  $SD = 0.22$ ) than during FTF ( $M = 0.40$ ,  $SD = 0.25$ ). The omnibus

analysis also indicated a significant main effect of risk-status,  $F(1, 48) = 6.10, p = 0.02$ . Across FTF and PAB interaction contexts, HR 6-month-olds spent a greater proportion of time looking toward ( $M = 0.63, SD = 0.21$ ) their mother than LR 6-month-olds ( $M = 0.48, SD = 0.19$ ).

*Looking away.* A final 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a main effect of interaction context,  $F(1, 48) = 41.14, p < 0.001$ . Overall, 6-month-olds looked away from their mother for a greater proportion of time during FTF ( $M = 0.42, SD = 0.23$ ) than PAB ( $M = 0.23, SD = 0.20$ ). No significant main or interaction effects of risk-status were found for this dependent measure.

***6-month analyses excluding HR-ASD infants.*** To assess whether risk group differences found in the full 6-month sample defined differences between LR and HR-no ASD infants, or were influenced by HR-ASD 6-month-olds within the HR group, behaviors that distinguished HR and LR infants in the full-sample analyses were reexamined using identical statistical tests and with the exclusion of HR-ASD infants. Five HR-ASD 6-month-olds were excluded from analyses. The series of repeated measures ANOVAs presented below reassess the influence of risk and interaction context on 6-month-olds' smiling, directed smiling, neutral affect, and looking toward mother. Change in findings would suggest that risk group differences reported in the above full-sample analyses were influenced by HR-ASD infants.

*Smiling.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context on smiling behavior,  $F(1, 43) = 46.78, p < 0.001$ . Overall, 6-month-olds of both risk groups spent a greater proportion of time smiling in PAB ( $M = 0.60, SD = 0.26$ ) than FTF ( $M = 0.39, SD = 0.27$ ) contexts. This is consistent with findings from the full-sample analysis. In contrast, the significant interaction effect of risk-status and interaction context found in the full-sample analysis disappeared after removing HR-ASD 6-

month-olds,  $F(1, 43) = 0.84, p = 0.37$ . Thus, LR and HR-no ASD 6-month-olds displayed a similar amount of smiling. See Table 19 for means and standard deviations of smiling behavior for HR-ASD, HR-no ASD, and LR groups. See Figures 8 and 9 for scatterplot representation of FTF and PAB individual-level smiling data organized by HR-ASD, HR-no ASD, and LR.

*Directed smiling.* Findings from a 2 (risk-status) by 2 (interaction context) repeated measures ANOVA excluding HR-ASD 6-month-olds were both consistent and inconsistent with findings from the full-sample analysis. Like in full-sample analyses a main effect of interaction context was found,  $F(1, 43) = 102.99, p < 0.001$ . Overall, 6-month-olds directed smiling to their mothers for a greater proportion of time in the PAB context ( $M = 0.52, SD = 0.26$ ) than the FTF context ( $M = 0.23, SD = 0.18$ ). Inconsistent with findings from the full-sample analysis, however, a significant interaction of risk and context on infants' directed smiling was not found,  $F(1, 43) = 1.86, p = 0.18$ . That is, LR and HR-no ASD 6-month-olds directed their smiles similarly across interaction contexts. See Table 19 for means and standard deviations of directed smiling behavior for HR-ASD, HR-no ASD, and LR groups. See Figures 10 and 11 for scatterplot representations of FTF and PAB individual-level directed smiling data organized by HR-ASD, HR-no ASD, and LR.

*Neutral affect.* Like in full sample analyses, a 2 (risk-status) by 2 (interaction context) repeated measures ANOVA revealed a significant main effect of context on infants' displays of neutral affect,  $F(1, 43) = 40.56, p < 0.001$ . Overall, 6-month-olds displayed more neutral affect during FTF ( $M = 0.57, SD = 0.25$ ) than PAB ( $M = 0.38, SD = 0.25$ ). In contrast, the significant interaction of risk-status and interaction context found in the full-sample analysis disappeared upon removal of HR-ASD 6-month-olds,  $F(1, 43) = 2.85, p = 0.10$ . LR and HR-no ASD 6-month-olds displayed neutral affect similarly across interaction contexts. See Table 19 for

means and standard deviations of neutral affect for HR-ASD, HR-no ASD, and LR groups. See Figures 12 and 13 for a scatterplot of FTF and PAB individual-level neutral affect data organized by HR-ASD, HR-no ASD, and LR.

*Looking toward mother.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA excluding HR-no ASD infants indicated a significant main effect of interaction context,  $F(1, 43) = 197.38, p < 0.001$ , and risk-status,  $F(1, 43) = 7.02, p = 0.01$ . Overall, infants looked at their mother for a greater proportion of time during PAB ( $M = 0.73, SD = 0.22$ ) than FTF ( $M = 0.38, SD = 0.24$ ). In addition, HR-no ASD 6-month-olds looked at their mothers for a greater proportion of time ( $M = 0.64, SD = 0.21$ ) than LR 6-month-olds ( $M = 0.48, SD = 0.19$ ). These results are consistent with full sample analyses. See Figure 14 for a scatterplot of individual-level looking data organized by HR-ASD, HR-no ASD, and LR.

*Summary of findings at 6-months.* First, analyses indicated that the interaction context influenced infants' expression of affect and looking. All 6-month-olds smiled more, directed their smiles more, looked toward their mother more, and displayed less neutral affect during PAB than FTF. Second, risk-group differences in affective behavior and looking are apparent at 6 months of age. For some dependent measures, risk-group differences appear to be influenced by the HR-ASD 6-month-olds within the HR sample. For other dependent measures, risk-group differences distinguished a) LR 6-month-olds from the HR sample as a whole (HR-ASD and HR-no ASD infants), and b) LR and HR-no ASD 6-month-olds independently. Smiling, directed smiling, and neutral affect distinguished LR 6-month-olds and the HR group as a whole, but did not distinguish LR and HR-no ASD 6-month-olds. This suggests that group differences found in full-sample analyses were influenced by HR-ASD 6-month-olds. Visual examination of the average amount of smiling, directed smiling, and neutral affect displayed by HR-no ASD, HR-

ASD, and LR 6-month-olds confirm that, indeed, HR-ASD infants behaved differently than other groups (see Table 19). In contrast, increased looking to mother distinguished both the HR sample as a whole and HR-no ASD 6-month-olds from LR 6-month-olds. This suggests that increased looking may be a characteristic of general HR status and not necessarily a unique characteristic of ASD at 6 months of age. Overall, this series of analyses suggests that facial expression production (i.e., smiling, directed smiling, neutral affect) may be uniquely important to the definition of socio-emotional development of ASD at 6 months, while looking behavior is potentially important to the definition of HR status at 6 months.

***Full-sample analyses at 11-months.*** As at the 6-month age point, a series of 2 (risk-status) by 2 (interaction context) repeated measures ANOVAs were conducted to determine the effects of these independent variables on 11-month-olds' smiling, directed smiling, neutral affect, and looking toward and away from their mother. Findings for each dependent variable are described below.

***Smiling.*** A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA revealed a significant main effect of interaction context on smiling behavior at 11 months of age,  $F(1, 55) = 70.35, p > 0.001$ . On average, 11-month-olds smiled for a significantly greater portion of interaction time during PAB ( $M = 0.63, SD = 0.24$ ) than FTF ( $M = 0.32, SD = 0.23$ ). The analysis also indicated a main effect of risk-status, such that across interaction contexts HR 11-month-olds smiled for a significantly greater proportion of time ( $M = 0.54, SD = 0.20$ ) than LR 11-month-olds ( $M = 0.43, SD = 0.18$ ),  $F(1, 55) = 5.26, p = 0.03$ .

***Directed Smiling.*** A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context on directed smiles,  $F(1, 55) = 100.30, p < 0.001$ . Across risk groups, 11-month-olds directed smiles to their mother for a significantly

greater proportion of time in PAB ( $M = 0.55$ ,  $SD = 0.26$ ) than the FTF context ( $M = 0.20$ ,  $SD = 0.16$ ). The analysis also revealed a significant main effect of risk,  $F(1, 55) = 6.08$ ,  $p = 0.02$ .

Across interaction contexts, HR 11-month-olds spent a greater amount of time in directed smiling ( $M = 0.42$ ,  $SD = 0.19$ ) than LR 11-month-olds ( $M = 0.32$ ,  $SD = 0.14$ ).

*Crying.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context on the proportion of interaction time 11-month-olds spent crying,  $F(1, 55) = 7.00$ ,  $p = 0.01$ . Both HR and LR 11-month-olds spent a greater proportion of time crying in FTF ( $M = 0.11$ ,  $SD = 0.19$ ) than PAB ( $M = 0.06$ ,  $SD = 0.13$ ). No significant main or interaction effects of risk on crying behavior were found.

*Neutral affect.* A 2 (risk-status) by 2 (interaction context) repeated measure ANOVA indicated a significant main effect of interaction context on neutral affect,  $F(1, 55) = 74.97$ ,  $p < 0.001$ . Overall, 11-month-old infants displayed more neutral affect during FTF ( $M = 0.47$ ,  $SD = 0.20$ ) than PAB ( $M = 0.29$ ,  $SD = 0.20$ ). No significant main or interaction effects of risk status were found.

*Looking toward mother.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context on the proportion of time 11-month-olds spent looking toward their mother,  $F(1, 55) = 186.53$ ,  $p < 0.001$ . On average, 11-month-olds spent a greater proportion of interaction time looking toward mother during PAB ( $M = 0.73$ ,  $SD = 0.19$ ) than FTF ( $M = 0.40$ ,  $SD = 0.18$ ). No significant main or interaction effects of risk-status were found.

*Looking away.* A final 2 (risk-status) by 2 (interaction context) repeated measures ANOVA indicated a significant main effect of interaction context on the proportion of time 11-month-olds spent looking away from their mother,  $F(1, 55) = 92.86$ ,  $p < 0.001$ . Both HR and LR

11-month-olds looked away from their mother for a significantly greater proportion of FTF ( $M = 0.48$ ,  $SD = 0.16$ ) than PAB ( $M = 0.24$ ,  $SD = 0.18$ ). No significant main or interaction effects of risk-status were found.

***11-month analyses excluding HR-ASD infants.*** Below are the results of ANOVA analyses conducted for a second time with the exclusion of HR-ASD 11-month-olds. These analyses provide further information about whether dependent measures are more likely indicators of HR status or HR-ASD at 11 months. As at the 6-month time point, any change in results after exclusion of HR-ASD infants would suggest that risk group differences found in full sample analyses may be attributable to HR-ASD 11-month-olds.

*Smiling.* A 2 (risk-status) by 2 (interaction context) repeated measures ANOVA, excluding HR-ASD 11-month-olds, yielded results consistent with full-sample analyses. A significant main effect of interaction context was found,  $F(1, 51) = 64.04$ ,  $p < 0.001$ . That is, LR and HR-no ASD 11-month-olds spent a greater proportion of time smiling during PAB ( $M = 0.63$ ,  $SD = 0.24$ ) than during FTF ( $M = 0.32$ ,  $SD = 0.23$ ). Results also continued to indicate a significant main effect of risk-status,  $F(1, 51) = 3.91$ ,  $p = 0.05$ . HR-no ASD 11-month-olds smiled for a greater proportion of time ( $M = 0.53$ ,  $SD = 0.21$ ) than LR 11-month-olds ( $M = 0.43$ ,  $SD = 0.18$ ) across interaction contexts. See Figure 15 for a scatterplot of individual-level smiling data for LR, HR-no ASD, and HR-ASD 11-month-olds.

*Directed smiling.* Results of a 2 (risk-status) by 2 (interaction context) repeated measures ANOVA excluding HR-ASD 11-month-olds were again consistent with full sample analyses. A significant main effect of interaction context was revealed,  $F(1, 51) = 84.32$ ,  $p < 0.001$ , with all infants directing a greater proportion of their smiles to their mother during PAB ( $M = 0.53$ ,  $SD = 0.24$ ) than FTF ( $M = 0.19$ ,  $SD = 0.16$ ). There also continued to be a significant main effect of

risk,  $F(1, 51) = 5.45, p = 0.02$ . HR-no ASD 11-month-olds directed a greater proportion of their smiles to their mother ( $M = 0.42, SD = 0.19$ ) than LR 11-month-olds ( $M = 0.32, SD = 0.14$ ) across interaction contexts. See Figure 16 for a scatterplot of individual-level directed smiling data for LR, HR-no ASD, and HR-ASD 11-month-olds.

***Summary of findings at 11 months.*** First, results indicate that interaction context had a significant effect on all 11-month-olds' affective displays and looking behavior. That is, HR and LR 11-month-olds displayed more smiling, directed smiling, and looking to mother, but less crying, neutral affect, and looking away from mother during PAB than FTF. Second, significant risk group differences in affect, but not looking behavior exist at the 11 month age point. In FTF and PAB contexts HR 11-month-olds spend more time smiling and in directed smiling than LR 11-month-olds. Lastly, there were no changes in results following the exclusion of HR-ASD infants. This suggests that risk-group differences in affect at 11 months are best characterized as features of HR status, not features that define HR-ASD at 11 months.

### **3.1.4 Infants' Facial Affect and Looking as Predictors of Diagnostic Outcome**

The above analyses of HR and LR 6- and 11-month-olds' facial expression production and looking behavior provided a conceptual basis for determining which of these behaviors may be significant predictors of atypical development and, more specifically, ASD. Results of these analyses guided the next series of analyses. The next analyses utilized logistic regression to predict the probability of general atypical development and ASD diagnosis in toddlerhood from affective and looking behaviors measured at 6- and 11-months during mother-infant interaction.



The numbers of 6- and 11-month-olds classified in each diagnostic category at toddler diagnostic outcome time points are presented in Table 20 and 21, respectively.

Two types of logistic regression analyses were conducted utilizing experimental data collected through mother-infant interaction at each age point. The first type of regression analysis predicted *Atypical Development* from risk-status and behaviors that distinguished HR and LR 6- and 11-month-olds in prior ANOVA analyses. Infants categorized in the *Atypical Development* category were those who received any diagnostic classification other than *Typically Developing* at diagnostic outcome, including: *ASD*, *Social Concerns*, *Global Developmental Delay*, or *Language Delay without ASD*. The purpose of the logistic regression was to determine if variables, measured at 6 and 11 months, would distinguish those participants who fell into the category of *Atypical Development* from those categorized as *Typically Developing*. The second type of logistic regression was conducted to determine whether 6- and 11-month measures would distinguish those participants with *ASD* from participants in all other diagnostic outcome categories. Independent variables considered for inclusion in this type of regression analysis were risk-status and behavioral measures that appeared, based on prior ANOVA analyses of risk group differences, to distinguish HR and LR infants at 6 or 11 months of age.

***Logistic regression at 6 months.*** The following variables were considered for entry into logistic regression equations predicting *Atypical Development* and *ASD*:

- 1) infants' risk-status
- 2) percent duration of FTF spent overall smiling
- 3) percent duration of FTF spent in directed smiling
- 4) percent duration of FTF spent in neutral affect
- 5) percent duration of FTF spent looking toward mother

6) percent duration of PAB spent looking toward mother

These variables were considered conceptually appropriate because they a) have been utilized in prior infant sibling studies as predictors of diagnostic outcome (e.g., Ozonoff et al., 2010) and b) were shown to distinguish HR and LR 6-month-olds in ANOVA analyses performed as a part of the current study.

Before predictors were entered into logistic regression equations, inter-correlations among variables were examined in order to assess issues of multicollinearity. Variables denoting the percentage of time that infants spent looking toward their mother during FTF and PAB were significantly, positively correlated ( $r^2 = 0.69$ ,  $p = 0.00$ ), therefore a composite variable was created. Variables denoting the proportion of time infants spent in overall smiling and directed smiling during FTF were also significantly, positively correlated ( $r^2 = 0.81$ ,  $p = 0.00$ ), therefore a composite variable was created. No other variables were significantly, positively correlated, indicating a need for consideration of multicollinearity. After considering inter-correlations among variables the following were included as predictors in logistic regression analyses:

- 1) infants' risk-status (*risk*)
- 2) composite percent duration of FTF and PAB spent looking toward mother (*complookmom*)
- 3) percent duration of FTF spent in neutral affect (*neutral*)
- 4) composite percent duration of FTF and PAB spent in overall smiling and directed smiling (*compsmiling*)

*Predicting atypical development from 6 months.* Initially, a logistic regression analysis was conducted predicting the diagnostic classification of *Atypical Development* using risk-status, a composite of looking toward mother, neutral affect, and a composite of smiling behavior as

predictors. The following prediction equation shows how the dependent variable, *Atypical Development*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{complookmom} + b3*\textit{neutral} + b4*\textit{compsmiling}$$

A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between *Atypical Development* and *Typical Development*, (chi square = 20.18,  $p < 0.001$  with  $df = 4$ ). Prediction success overall was 84.4% (97.1% for *Typical Development* and 60.0% for *Atypical Development*). However, preliminary analyses indicating whether each independent variable improves the model found that only risk-status, neutral affect, and the smiling composite contributed to the prediction of *Atypical* versus *Typical Development* classification in a significant manner. The looking toward mother composite variable was not determined to significantly contribute to the model, and therefore, was removed as a predictor.

A new prediction equation was developed, excluding the composite variable of looking to mother. The equation below shows how the dependent variable, *Atypical Development*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{neutral} + b3*\textit{compsmiling}$$

A test of the full model against a constant only model was again statistically significant, indicating that the predictors as a set reliably distinguished between *Atypical Development* and *Typical Development*, (chi square = 9.81,  $p = 0.02$  with  $df = 3$ ). Prediction success overall was 84.1% (94.3% for *Typical Development* and 44.4% for *Atypical Development*). Wald criterion demonstrated that none of these independent variables made a significant contribution to the prediction of diagnostic classification. The log-odds values, significant level of these values, and odd ratio values of each predictor are presented in Table 22.

*Predicting ASD diagnosis from 6 months.* Next, a logistic regression analysis was conducted predicting the diagnostic classification of *ASD* with risk-status, a composite looking toward mother variable, neutral affect, and a composite smiling behavior variable considered as potential predictors. The following prediction equation displays how the dependent variable, *ASD*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{complookmom} + b3*\textit{neutral} + b4*\textit{compsmiling}$$

A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between *ASD* and all other diagnostic outcome classifications (chi square = 17.29,  $p < 0.001$  with  $df = 4$ ). Prediction success overall was 90.9% (94.9% for other diagnostic classifications and 60.0% for *ASD*). Preliminary analyses assessing whether each independent variable improves the model found that only risk-status, neutral affect, and the smiling composite variable significantly contributed to the prediction of *ASD* versus other diagnostic classification. The looking toward mother composite variable was not determined to significantly contribute to the model, and therefore, was excluded from the logistic regression equation.

A new prediction equation, excluding the composite variable of looking to mother, was developed. The equation below shows how the dependent variable, *ASD*, was predicted from the remaining independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{neutral} + b3*\textit{compsmiling}$$

A test of the full model against a constant only model was again statistically significant, indicating that the predictors as a set reliably distinguished between *Atypical Development* and *Typical Development*, (chi square = 9.11,  $p = 0.03$  with  $df = 3$ ). Prediction success overall was 88.6% (100% for other diagnostic classifications and 0% for *ASD*). Wald criterion demonstrated

that none of the independent variables made a significant contribution to prediction. The log-odds values, significant level of these values, and odd ratio values of each predictor are presented in Table 22.

***Summary of results at 6 months.*** Neither logistic regression analysis attempting to predict classification of *Atypical Development* versus *Typical Development*, or *ASD* versus any other diagnostic outcome found any independent variable measured at 6 months of age to be a significant predictor of diagnostic classification.

***Logistic regression at 11 months.*** Based on findings of risk group differences at 11-months, the following variables were considered for entry into logistic regression equations predicting *Atypical Development* and *ASD*:

- 1) infants' risk-status
- 2) percent duration of FTF spent smiling
- 3) percent duration of PAB spent smiling
- 4) percent duration of FTF spent in directed smiling
- 5) percent duration of PAB spent in directed smiling

Before predictors were entered into logistic regression equations, inter-correlations among variables were examined to address issues of multicollinearity. Significant, positive correlations were found among all smiling variables (see Table 23 for  $r^2$  values and significance levels). Therefore, to address the issue of shared variance, a composite of all smiling variables was created. The new variables considered for entry into logistic regression equations predicting *Atypical Development* and *ASD* were:

- 1) infants' risk-status (***risk***)
- 2) composite of all smiling variables (***compsmiling***)

*Predicting atypical development from 11 months.* A logistic regression analysis was conducted predicting the diagnostic classification of *Atypical Development* using risk-status and a composite of 11-month smiling behavior as predictors. The following prediction equation shows how the dependent variable, *Atypical Development*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{compsmiling}$$

A test of the full model against a constant only model was not statistically significant, indicating that the predictors as a set did not reliably distinguish between *Atypical Development* and *Typical Development*, (chi square = 3.87,  $p = 0.14$  with  $df = 2$ ). In addition, preliminary analyses assessing whether each independent variable improves the model found that neither risk-status nor the smiling composite variable significantly contributed to the prediction. Based on these preliminary findings, which indicated that the model lacked sufficient goodness of fit, the planned logistic regression analysis was not completed.

*Predicting ASD diagnosis from 11 months.* Next, a logistic regression was conducted predicting the diagnostic classification of ASD versus all other diagnostic outcomes using risk-status and a composite of 11-month smiling behavior as predictors. The following prediction equation displays how the dependent variable, *ASD*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textit{risk} + b2*\textit{compsmiling}$$

In this case, a test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between ASD and other diagnostic classifications. Preliminary analyses assessing whether each independent variable improves the model found that risk-status, but not the smiling composite variable, significantly

contributed to the prediction. Removing the smiling composite variable, the new prediction equation was as follows:

$$\log(p/1-p) = b0 + b1*\textit{risk}$$

A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between ASD and other diagnostic outcomes, (chi square = 8.13,  $p = 0.00$  with  $df = 1$ ). Prediction success overall was 90% (100% for other diagnostic classifications and 0% for ASD). Wald criterion demonstrated that the independent variable of risk-status did not make a significant contribution to prediction. The log-odds values, significant level of these values, and odd ratio values of this predictor are presented in Table 24.

*Summary of results at 11 months.* Neither logistic regression analysis predicting classification as atypically developing versus typically developing, or ASD versus any other diagnostic outcome, found any of the independent variable measure at 11 months of age to be a significant predictor of diagnostic outcome. In fact, at the 11 month age point, prediction models including experimentally measured predictors were not found to be sufficient to move beyond preliminary analyses evaluating model goodness of fit.

## **3.2 EYE-TRACKING PROCEDURE ASSESSING EMOTION PERCEPTION**

### **3.2.1 Preliminary Analyses**

Analyses of data from the eye-tracking procedure assessing emotion perception began with preliminary examination of descriptive data for each dependent measure. Dependent measures

included: a) total duration looking to the total stimulus area, b) ratio of looking to the smiling face/total stimulus area, c) ratio of looking to the total face area/total stimulus area (i.e., on-off ratio), d) ratio of looking to inner facial features/inner + outer facial features (i.e., inner-outer ratio), and e) ratio of looking to the eye region/eye + mouth region (i.e., eye-mouth ratio). The mean, standard deviation, and range of values for each variable organized by smile intensity type are presented for HR and LR 6- and 11-month-olds in Tables 25 and 26, respectively.

An additional analysis investigated potential gender differences in infants' looking to smile/neutral face pairs. A multivariate ANOVA, with gender as a between-subjects variable, was conducted to assess whether male and female 6- and 11-month-olds differed on any dependent measure. No significant gender differences were found on any variable; therefore, infants' gender was not included as a covariate in subsequent analyses.

### **3.2.2 Infants' Visual Attention to Emotion as a Marker of HR**

Results of analyses examining risk group differences in visual attention to smiling/neutral face pairs at 6 months of age are presented first, followed by results of analyses conducted at the 11-month age point. At each age point, results of full-sample analyses are presented first, followed by results from analyses excluding HR-ASD infants. Only dependent measures that indicated a significant risk effect in full-sample analyses were reassessed by repeating identical ANOVA analyses with the exclusion of HR-ASD infants. A summary of ANOVA results conducted for the 6 and 11 month sample is presented in Tables 27 and 28, respectively.

***Full-sample analyses at 6-months.*** Separate 2 (risk-status) x 3 (smile intensity) repeated measures ANOVAs were conducted to assess the impact of risk-status and smile intensity on a) the total time infants spent looking at the total stimulus area, b) the ratio of looking to the smiling



face/total stimulus area, c) the ratio of looking to the total face area/total stimulus area (i.e., on-off ratio), d) the ratio of looking to inner facial features/inner + outer facial features (i.e., inner-outer ratio), and e) the ratio of looking to the eye region/eye + mouth region (i.e., eye-mouth ratio). Findings are presented by dependent measure.

*Total stimulus looking.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA was conducted to assess the influence of these variables on the total time 6-month-olds spent looking at smile/neutral face pair stimuli. The analysis revealed a significant main effect of risk-status only,  $F(1,57) = 7.42, p = 0.01$ . That is, HR 6-month-olds spent a significantly greater amount of time looking at smile/neutral stimuli ( $M = 54.03, SD = 14.98$ ) than LR 6-month-olds ( $M = 41.37, SD = 20.53$ ). There were no significant main or interaction effects of smile intensity.

*Looking to the smiling face.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA was conducted to assess the impact of these variables on the ratio of looking time spent attending to the half of the stimulus that held the smiling face/the total stimulus area. Analyses revealed a significant main effect of smiling intensity only,  $F(2, 56) = 7.14, p = 0.002$ . Follow-up analyses indicated that 6-month-olds looked at the smiling half of the stimulus for a greater proportion of stimulus looking time when the smiling face was most exaggerated (i.e., jaw-drop smile;  $M = 0.56, SD = 0.12$ ), in comparison to when the smiling face was least exaggerated (i.e., closed-mouth smile;  $M = 0.48, SD = 0.11$ ),  $t(58) = -3.80, p < 0.001$ . In addition, 6-month-olds looked at the smiling half of the stimulus for a greater proportion of stimulus looking time when the smiling face was most exaggerated ( $M = 0.56, SD = 0.12$ ), in comparison to when the smiling face displayed a mid-level, or prototypical smile ( $M = 0.51, SD = 0.12$ ),  $t(58) = -2.51, p = 0.02$ . See Figure 2 for an example of smile/neutral pairings at each intensity level.

*Looking to the face area.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA was conducted to assess the impact of these variables on the ratio of looking time spent attending to the face portion of the stimulus. No significant main or interaction effects of risk-status or smiling intensity were found.

*Looking to the inner features of the face.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA was conducted to assess the influence of these variables on the ratio of looking time 6-month-olds spent attending to the inner facial features of the face. This analysis indicated a significant main effect of risk-status,  $F(1, 57) = 7.20, p = 0.01$ . HR 6-month-olds looked at the internal features of the face for a significantly greater proportion of total face looking time ( $M = 0.66, SD = 0.13$ ) than LR 6-month-olds ( $M = 0.59, SD = 0.12$ ). In addition, a significant main effect of smiling intensity was revealed,  $F(2, 56) = 6.66, p = 0.003$ . Across risk groups, 6-month-olds looked at the internal features of the face for a greater proportion of total face-looking time when the smile/neutral pairing included a low intensity smile (i.e., closed-mouth smile;  $M = 0.64, SD = 0.16$ ), in comparison to when the smile/neutral pairing included a prototypical, or mid-intensity smile ( $M = 0.57, SD = 0.16$ ),  $t(58) = 2.96, p < 0.001$ . Additionally, 6-month-olds looked at the internal features of the face for a greater proportion of total face-looking time when the smile/neutral pairing included the highest intensity smile (i.e., jaw-drop smile;  $M = 0.66, SD = 0.20$ ), in comparison to when the smile/neutral pairing included a prototypical smile ( $M = 0.57, SD = 0.16$ ),  $t(58) = -3.30, p < 0.001$ .

*Looking to the eye versus mouth region.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA indicated a significant main effect of smile intensity on the ratio of looking time 6-month-olds spent attending to the eye region versus total eye/mouth region of the face,

$F(2, 56) = 15.59, p < 0.001$ . Across risk groups, 6-month-olds looked to the eye region for a greater proportion of eye/mouth looking time when the smile/neutral pairing included a face displaying a low intensity smile ( $M = 0.74, SD = 0.28$ ), in comparison to when the smile/neutral pairing included a face displaying a prototypical, mid-intensity smile ( $M = 0.56, SD = 0.38$ ),  $t(58) = 4.65, p < 0.001$ . In addition, 6-month-olds attended to the eye region of the face for a greater proportion of eye/mouth looking time when the smile/neutral pairing included a face displaying a low intensity smile ( $M = 0.74, SD = 0.28$ ), in comparison to when the smile/neutral pairing included a high-intensity, exaggerated smile ( $M = 0.54, SD = 0.37$ ),  $t(58) = 4.89, p < 0.001$ .

***6-month analyses excluding HR-no ASD infants.*** To assess whether risk group differences found in analyses of the full sample were influenced by HR-ASD infants, looking variables that distinguished HR and LR 6-month-olds in full-sample analyses (i.e., total stimulus looking and looking to the inner features of the face) were reexamined excluding the three HR-ASD infants within the HR 6-month-old group.

*Total stimulus looking.* As in full-sample analyses, a 2 (risk-status) by 3 (smiling intensity) repeated measures ANOVA excluding HR-ASD infants indicated a significant main effect of risk-status,  $F(1, 54) = 6.79, p = 0.01$ . HR-no ASD 6-month-olds displayed significantly more looking to the total stimulus area ( $M = 53.76, SD = 14.54$ ) than their LR counterparts ( $M = 41.37, SD = 20.52$ ),  $t(54) = 2.61, p = 0.03$ . In addition, and consistent with full-sample analyses, no significant main or interaction effects of smile intensity were found.

*Looking to the inner features of the face.* Results of a 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA excluding HR-ASD 6-month-olds were consistent with full-sample analyses. HR-no ASD 6-month-olds spent a greater proportion of their total looking

time attending to the inner features of the face ( $M = 0.67$ ,  $SD = 0.13$ ) than their LR counterparts ( $M = 0.59$ ,  $SD = 0.12$ ),  $F(1, 54) = 7.11$ ,  $p = 0.01$ . Also consistent with full-sample analyses ANOVA analyses revealed no main or interaction effects of smile intensity.

***Summary of findings at 6 months.*** Findings indicate significant risk-group differences in the a) total amount of time spent looking to stimuli during the eye tracking paradigm and b) proportion of total looking time 6-month-olds spent attending to the internal features of the face. The full sample of HR 6-month-olds, as well as the subset of HR-no ASD 6-month-olds, spent a significantly greater amount of time looking at smile/neutral face pairing stimuli and looking to the inner features of the face than LR 6-month-olds (see Table 29 for group means and standard deviations). This indicates that differences in looking may define general HR status. In addition, results indicate a significant effect of smile intensity on looking behavior across risk groups. All 6-month-olds looked significantly longer at the side of the stimulus displaying the smiling face when the smile was most exaggerated in comparison to when the smile displayed was a prototypical smile and a low intensity smile. Infants also looked at the internal features of the face most when the smile included in the smile/neutral pairing was at the extreme intensity levels, either the highest intensity or lowest intensity smile. Finally, 6-month-olds looked longer at the eyes than mouth when smile/neutral pairs included a low intensity smile, than when smile/neutral pairs included a prototypical or high intensity smile.

***Full-sample analyses at 11-months.*** Separate 2 (risk-status) x 3 (smile intensity) repeated measures ANOVAs were conducted to assess differences in 11-month-olds' visual attention based on risk-status and stimulus smile intensity. Dependent measures are identical to those assessed at 6 months. Findings for each dependent variable are presented below.

*Total stimulus looking.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA revealed a significant main effect of smile intensity on the total time 11-month-olds spent looking at smile/neutral face pair stimuli,  $F(2, 66) = 4.30, p = 0.02$ . Follow-up analyses indicate that 11-month-olds looked longer at stimuli that included a high intensity, exaggerated smile ( $M = 15.15, SD = 5.83$ ) than those that included a low intensity smile ( $M = 13.69, SD = 5.38$ ),  $t(68) = -2.47, p = 0.02$ ; or a prototypical, mid-intensity smile ( $M = 13.53, SD = 4.90$ ),  $t(68) = -2.80, p = 0.01$ . No significant main or interaction effects of risk-status were found.

*Looking to the smiling face.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA indicated a significant main effect of smiling intensity on the ratio of total stimulus looking time 11-month-olds spent attending to the half of the stimulus that held the smiling face,  $F(2, 66) = 7.48, p = 0.001$ . Across risk groups, 11-month-olds looked at the half of the stimulus that held the smiling face for a greater portion of total stimulus looking time when the smile displayed a high-intensity, exaggerated smile ( $M = 0.57, SD = 0.10$ ), in comparison to when the smile displayed a low-intensity smile ( $M = 0.51, SD = 0.10$ ),  $t(68) = -3.90, p < 0.001$ ; or when the smile displayed a prototypical, mid-intensity smile ( $M = 0.53, SD = 0.01$ ),  $t(68) = -2.11, p = 0.04$ . No significant main or interaction effects of risk-status were found.

*Looking to the face area.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA indicated a significant main effect of smile intensity on the ratio of looking time spent attending to the face portion of the stimulus,  $F(2, 66) = 5.44, p = 0.007$ . Across risk groups, 11-month-olds looked at the face portion of the stimulus for a greater proportion of time when the stimulus included a high-intensity, exaggerated smile ( $M = 0.79, SD = 0.15$ ), in comparison to when the smile displayed a low-intensity smile ( $M = 0.75, SD = 0.16$ ),  $t(68) = -2.50, p = 0.02$ ; or

when the smile displayed a prototypical, mid-intensity smile ( $M = 0.75$ ,  $SD = 0.16$ ),  $t(68) = -3.16$ ,  $p < 0.001$ . No significant main or interaction effects of risk-status were found.

*Looking to the inner features of the face.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA revealed a significant main effect of smile intensity on the ratio of looking time 11-month-olds spent attending to the inner facial features of the face,  $F(2, 66) = 8.0$ ,  $p = 0.001$ . Across risk groups, a greater proportion of time was spent attending to the inner features of the face when 11-month-olds were presented with a smile/neutral pair that included a high-intensity smile ( $M = 0.61$ ,  $SD = 0.19$ ), than when presented with a smile/neutral pair that included a low-intensity smile ( $M = 0.55$ ,  $SD = 0.20$ ),  $t(68) = -2.49$ ,  $p = 0.02$ ; or when presented with a smile/neutral pairing with a mid-intensity smile ( $M = 0.53$ ,  $SD = 0.13$ ),  $t(68) = -3.97$ ,  $p < 0.001$ . No significant main or interaction effects of risk-status were found.

*Looking to the eye versus mouth region of the face.* A 2 (risk-status) x 3 (smiling intensity) repeated measures ANOVA indicated a significant main effect of smile intensity on the ratio of looking time 11-month-olds spent attending to the eye region versus total eye/mouth region of the face,  $F(2, 66) = 4.88$ ,  $p = 0.01$ . Across risk groups, a greater proportion of eye-mouth looking time was spent attending to the eye features of the face when 11-month-olds were presented with a smile/neutral pair that included a low-intensity smile ( $M = 0.43$ ,  $SD = 0.37$ ), than when presented with a smile/neutral pair that included a high-intensity smile ( $M = 0.34$ ,  $SD = 0.31$ ),  $t(68) = 3.01$ ,  $p < 0.001$ . No significant main or interaction effects of risk-status were found.

***11-month analyses excluding HR-no ASD infants.*** Because no significant main or interaction effects of risk-status were found in full-sample analyses, further statistical tests that excluded HR-ASD infants were not conducted.

***Summary of findings at 11 months.*** For each visual attention variable a main effect of smile intensity was found, but no significant risk group differences in visual attention were observed. Infants looked longer at the smile/neutral face stimuli when the pairing included a high intensity smile than when the pairing included a prototypical or low intensity smile. Infants at this age also spent a greater proportion of their overall looking time looking at the smile half of the face, the face area, and the internal features of the face when the smile was exaggerated, in comparison to when the smile was prototypical or low intensity. In contrast, 11-month-olds looked longer at the eye than mouth region of the face when the smile/neutral pairing included a low intensity smile in comparison to when the smile was high intensity.

### **3.2.3 Infants' Visual Attention to Emotion as Predictors of Diagnostic Outcome**

The above analyses of HR and LR infants' visual attention to smile/neutral face pairs provided a conceptual basis for determining which looking variables may be significant, early predictors of atypical development and, more specifically, ASD. Results of analyses examining risk group differences guided the next series of analyses which utilized logistic regression to predict atypical development and ASD diagnosis. Those looking behaviors that were performed differently by HR and LR infants were considered for inclusion in regression analyses. Since above analyses of risk group differences indicated that none of the looking variables assessed distinguished HR and LR infants at 11 months, the following logistic regression analyses were only conducted to predict outcome from the 6-month age point. The number of 6-month-olds classified in each diagnostic category is presented in Table 8.

Two logistic regression analyses were conducted. The first considered risk-status and looking behaviors that distinguished HR and LR 6-month-olds as potential predictors of *Atypical*

*Development* in toddlerhood. The second logistic regression was conducted to determine whether risk-status and looking behaviors that distinguish HR and LR 6-month-olds are unique predictors of *ASD* diagnosis in toddlerhood. The purpose of these logistic regression analyses was to determine if variables measured at 6 months would distinguish: a) those participants who fell into the category of *Atypical Development* from those categorized as *Typically Developing*, and b) those participants with *ASD* from participants with any other diagnosis. Infants categorized in the *Atypical Development* category were those who received any diagnostic classification other than *Typically Developing* at diagnostic outcome, including: *ASD*, *Social Concerns*, *Global Developmental Delay*, or *Language Delay without ASD*.

***Logistic regression at 6 months.*** The following variables were considered for entry into logistic regression equations predicting *Atypical Development* and *ASD*:

- 1) infants' risk-status (***risk***)
- 2) duration of looking to the total stimulus area (***totalstimlook***)
- 3) percent duration of looking to the internal features of smile/neutral faces  
(***internallook***)

These variables were considered conceptually appropriate because they were shown to distinguish HR and LR 6-month-olds in ANOVA analyses performed as a part of the current study.

Before predictors were entered into logistic regression equations, inter-correlations among variables were examined in order to assess issues of multicollinearity. None of the variables were significantly, positively correlated, indicating a need for further consideration of multicollinearity. Thus, all the above noted variables were included as predictors in logistic regression analyses.



*Predicting atypical development from 6 months.* First, a logistic regression analysis was conducted predicting the diagnostic classification of *Atypical Development*. Predictors included: risk-status, duration of looking to the total stimulus area, and percent duration of looking to the internal features of smile/neutral face pairs. The following prediction equation shows how the dependent variable, *Atypical Development*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textbf{risk} + b2*\textbf{totalstimlook} + b3*\textbf{internallook}$$

A test of the full model against a constant only model was not statistically significant, indicating that the predictors as a set do not reliably distinguish between *Atypical Development* and *Typical Development*, (chi square = 6.68,  $p = 0.08$  with  $df = 3$ ). Prediction success overall was 78.7% (100% for *Typical Development* and 13.3% for *Atypical Development*). Preliminary analyses indicating whether the addition of each independent variable improves the model fit found that while risk-status contributed to the prediction of *Atypical* versus *Typical Development* classification in toddlerhood, neither looking variable made a significant contribution. Because these two experimental variables of interest added no predictive value to the logistic regression analysis and because the model did not appear to be a good fitting model (Mernard, 2002), the logistic regression analysis was terminated at the preliminary analysis stage.

*Predicting ASD diagnosis from 6 months.* Next, a logistic regression analysis was conducted predicting the diagnostic classification of *ASD*. Predictors included risk-status, duration of total looking time to the total stimulus area, and the percent of total looking time spent looking to the internal features of the smile/neutral faces. The following prediction equation displays how the dependent variable, *ASD*, was predicted from these independent variables:

$$\log(p/1-p) = b0 + b1*\textbf{risk} + b2*\textbf{totalstimlook} + b3*\textbf{internallook}$$

A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between *ASD* and all other diagnostic outcome classifications (chi square = 8.92,  $p = 0.03$  with  $df = 3$ ). Prediction success overall was 88.5%. However, while the model successfully predicted 100% of cases falling into other, non-*ASD* diagnostic classifications, it did not successfully predict any cases that fell into the *ASD* category. Preliminary analyses assessing whether each independent variable improves the model fit also found that only risk-status, but neither looking variable, significantly contributed to the prediction of *ASD* versus other diagnostic classification. Because the experimental variables of interest added no predictive value to the logistic regression analysis, and because the model did not appear to correctly classify any cases with *ASD*, the model was determined to be a poorly fitted model (Mernard, 2002) and the logistic regression analysis was terminated at the preliminary analysis stage.

***Summary of findings at 6 months.*** Preliminary analysis of logistic regression equations, utilizing risk-status and visual attention variables measured at 6 months as predictors of a) atypical development and b) *ASD* did not indicate that either prediction model was a good fitting model. For this reason analyses were terminated at the preliminary stage.

## **4.0 DISCUSSION**

The current study explored the facial expression production and perception abilities that emerge in infants at HR and LR for ASD during the first year of life (i.e., 6 and 11 months). A primary aim was to draw conclusions about the quality or nature of these abilities. More specifically, this study sought to determine: a) how facial expression production and perception may emerge differently for HR and LR groups during the first year of life; b) whether behaviors that differentiated HR and LR groups could be considered characteristics of HR status or indicators of ASD; and c) whether the specific behaviors that differentiated risk groups and emerged as possible indicators of ASD changed with age. An additional aim of the current study was to make inferences about how emotion perception and production develop in tandem for HR and LR groups. A final aim was to consider the influence of contextual variables, such as mother-infant interaction type, the affective displays of infants' mothers, and the intensity of smiles the infant viewed. Consideration of context was incorporated into this study in an attempt to understand infant siblings' emotion development as a transactional process.

Findings related to facial expression production for both mother and infant will be considered first. This discussion is followed by consideration of visual attention to facial expressions as measured through the eye-tracking paradigm. For facial expression production and perception the following discussion will a) summarize findings regarding risk-group

differences and predictors of diagnostic outcome, and b) describe the role of context (i.e., mother's affect, interaction type, and smile intensity) in interpretation of findings. In a final segment of this discussion, results related to facial expression production and perception will be integrated, considered in the context of the current literature, and described in relation to experience-expectant model of emotion development and transactional models of development.

#### **4.1 ASSESSING EMOTION PRODUCTION**

In the present study the facial expression production and looking behavior of HR and LR 6- and 11-month-olds, and the affective expressions of these infants' mothers, was examined in the context of mother-infant interaction. Five general conclusions can be drawn from observations made of infants' and mothers' behavior. First, infants' affective and looking behaviors differ across different types of mother-infant interactions. Regardless of age or risk-status, infants appeared more affectively and visually engaged with their mother by PAB than FTF. Second, mother's affect differed based on their infants' age and the type of face-to-face interaction, but not based on their infant's risk-status. Mother's displayed more positive affect during interaction with 6- than 11-month-olds and more exaggerated positive affect during PAB than FTF, but HR and LR infants' mothers did not differ in their expression of affect. Third, facial expression and looking distinguish HR from LR infants throughout the first year of life – as early as 6 months and continuing to 11 months. Fourth, the pattern of affective and looking behavior that distinguishes HR and LR infants appears to change across the first year of life. Lastly, distinct patterns of facial expression production and looking characterize HR infants, distinguish HR-no

ASD infants from LR infants, and may distinguish HR-ASD infants from HR-no ASD and LR infants. In the next paragraphs, each of these general conclusions is considered in more detail.

#### **4.1.1 Consideration of Interaction Context**

Mothers' affect, as well as infants' affect and looking behavior, were influenced by mother-infant interaction type. Mothers displayed more frequent, exaggerated smiles during PAB than FTF, no matter the age or risk-status of their infant. Infants also displayed more affective and visual engagement in PAB than FTF. That is, no matter their age or risk status, infants displayed more smiling, directed more smiling, and looked more at their mother during PAB than FTF. Infants also displayed more neutral affect and looked away from their mother more during FTF than PAB. At 11 months, infants cried more during FTF than PAB. Taken together, these findings indicate that both mother and infant were more affectively and visually engaged during PAB than FTF. These findings are consistent with past research that indicates PAB elicits positive affect for both mother and infant (e.g., Sroufe & Waters, 1976; Washburn, 1929) and show that PAB elicits a level of affective and visual engagement over and above what is typical for less structured, normative FTF.

#### **4.1.2 Consideration of Mothers' Affect**

The present infant sibling study is unique in its assessment of mothers' *and* infants' behavior. No prior infant sibling studies of affect and looking behavior, measured in the context of face-to-face interaction, have measured the adult interaction partner's behavior. This is surprising

because research exploring typically developing infants' affect and looking indicates bidirectional influence between mother and infant behavior during FTF interaction (Cohn & Tronick, 1988; see Tronick, 1989 for a review). In fact, affective behaviors of mothers and typically developing infants are defined by periods of synchronicity (e.g., Feldman, Greenbaum, & Yirmiya, 1999), with increasing affective coordination and matching emerging across the first year of life (e.g., Tronick & Cohn, 1989).

Because mother and infant behaviors typically have bidirectional effects, it is important that infant sibling studies of facial expression production measure both infants' and mothers' behavior during mother-infant interaction. These considerations are important because of parents' awareness of, and sometimes concern about, the HR status of their infant. Mother's knowledge of their infants' risk and potential for disability can impact their level of stress, and parental stress has been shown to influence parenting behavior (e.g., Crnic, Greenberg, Ragozin, Robinson, & Basham, 1983; Halpern, Brand, & Malone, 2000). Thus, knowledge of risk could alter mother's affective behavior in interaction with their infant, in which case infant-specific risk-group differences in social emotional behavior would need to be considered in the context of co-occurring risk-group differences in mother's behavior. The current study finds that mother's affective behavior does not differ based on the risk-status of the infant. Having completed an assessment of mother's behavior, risk-group differences in infants' behavior can confidently be discussed as attributable to the infant and not risk-specific differences in mother's affect.

Mothers' affect did not differ by infant risk-status, but mother's affective displays did differ by infant's age. Mothers displayed more frequent, high intensity smiles when infants were 6 months than when they were 11 months of age. Thus, as infants developed through the first year of life, mothers displayed less exaggerated positive affect.

There are a number of possible explanations for this finding. First, mothers may provide less affective scaffolding at 11 months than at 6 months, allowing their infant to lead the interaction more as they get older. Second, change in mothers' behavior may result from infants' decreased responsiveness to face-to-face interaction across the first year of life. Social-action games, like PAB, are of less interest and are engaged in less often by mother-infant dyads as an infant develops beyond 8 months (e.g., Crawley, Rogers, Friedman, Iacobbo, Criticos, et al., 1978). Since infant behavior was analyzed cross-sectionally in the present study this explanation could not be tested, but may be explored in future investigations.

#### **4.1.3 Consideration of Infants' Facial Expressions and Looking**

The current study revealed group differences in HR and LR infants' affect and looking displays during the first year of life. Some affect and looking variables distinguish HR-no ASD infants from LR infants, indicating behaviors that may mark HR status. Other affect and looking behaviors may uniquely characterize HR-ASD infants. Thus, affect production and looking measured in a mother-infant interaction context may distinguish HR-no ASD, HR-ASD, and LR. In addition, some risk group difference in affect and looking behavior changed from 6 to 11 months of age and as a function of face-to-face interaction type (i.e., FTF or PAB). This suggests possible age-based change in behaviors that distinguish the groups, and highlights the importance of contextual factors. Below, these findings are further discussed for 6-month-old infants, then for 11-month-olds.

***6-month-olds' affect and looking.*** At 6 months, HR infants spent a large proportion of interaction time smiling and the amount of smiling produced remained unchanged across FTF

and PAB contexts. LR 6-month-olds only matched the high degree of smiling displayed by HR 6-month-olds in the PAB context, spending significantly more time smiling in the context of PAB than FTF. Directed smiles were also displayed differently by HR and LR 6-month-olds. In the context of FTF, HR 6-month-olds spent more time in directed smiling than LR 6-month-olds, but HR and LR 6-month-olds spent similar amounts of time in directed smiling during PAB. A final difference between HR and LR 6-month-olds was in the amount of time spent looking to their mother. Across FTF and PAB, HR 6-month-olds spent a greater portion of interaction time looking at their mother than LR 6-month-olds.

Taken together, these data present a specific picture of the HR 6-month-old. Increased levels of overall smiling behavior in FTF, with no change in smiling from FTF to PAB, suggests that HR 6-month-olds may be more highly and easily aroused in social interaction than the LR 6-month-old. HR 6-month-olds also spend more time looking toward mother than LR 6-month-olds across contexts. This means that HR 6-month-olds spent less time in visual disengagement from their mother, who is a highly arousing stimulus in their environment. With the combination of increased overall smiling and looking to mother in the FTF context, it is not surprising that HR 6-month-olds also displayed more directed smiling (i.e., the combination of smiles and looks to mother) during FTF than LR 6-month-olds. Thus, it is possible that risk-group differences in directed smiles are merely a product of observed increases in HR 6-month-olds overall smiling and looking to mother, and that these smiles do not have the same social communicative function that directed smiles are assumed to have for typically developing infants.

Although limited in number, prior infant sibling studies help explain increased smiling and increased looking toward mother displayed by HR 6-month-olds in the current study. Some of the earliest accounts of difference between HR and LR infants report that HR infants have



more difficulty visually disengaging from stimuli than LR infants. Disengaging attention involves the shifting of visual attention from one object to re-engage with another. The ability to disengage is an aspect of the visual system that typically develops between 1 and 4 months of age (e.g., Butcher, Kalverboer, Geuze, 2000; Johnson, Posner, & Rothbart, 2007), with infants ability to visually disengage appearing similar to that of adults by 6 months (e.g., Hood & Atkinson, 1993). HR infants show difficulty with visual disengagement at and after 6 months. In the context of social interaction, HR 6-month-olds shifted their gaze to and from their caregiver's face less frequently than LR 6-month-olds (Ibanez, Messinger, Newell, Lambert, & Sheskin, 2008). In the context of a looking paradigm, HR 9-10 month olds showed poorer ability to disengage from a central stimulus to look at a co-occurring peripheral stimulus than their LR counterparts (Elsabbagh, Volein, Holmboe, et al., 2009). Difficulty disengaging from stimuli in the environment could lead to increased looking to mother during face-to-face interaction. A HR infant with difficulty disengaging could appear to be visually "stuck" looking at their mother. In the current study, HR 6-month-olds' pattern of increased visual attention to mother may be related to difficulty with this aspect of visual system development. This explanation of current findings is consistent with prior studies that have described HR infants' visual attention as "sticky" (e.g., Zwaigenbaum et al., 2005).

Difficulty disengaging also provides a possible explanation for the current study finding that HR 6-month-olds display a consistent, high level of smiling across FTF and PAB contexts that is not displayed by LR 6-month-olds. During the first year of life typically developing infants develop the ability to visually disengage from their mother during face-to-face interaction (e.g., Brazelton, Tronick, Adamson, Als, & Wise, 1975; Cohn & Tronick, 1989). Visual engagement-disengagement cycles coincide with the ebb and flow of smiling behavior

(Messinger & Fogel, 2007). Visual disengagement in the context of face-to-face interaction is thought to assist in infants' ability to regulate heightened arousal that comes from the stimulation of face-to-face play (Fogel, 1993; Field, 1981; Stifter & Moyer, 1991; Tronick, 1989). If infants do not visually disengage from an arousing stimulus (i.e., their mother) in face-to-face play, they may display evidence of increased arousal. Consistently high amounts of smiling could be an indicator of increased arousal (Field, 1981; Sroufe & Water, 1976). Thus, in the current study, increased smiling by HR 6-month-olds in FTF interaction, which remains when the interaction context shifts to PAB, could be an indicator of over-arousal. Furthermore, over-arousal may be explained, in part, by difficulty visually disengaging from a highly arousing stimulus (i.e., mother).

Prior research assessing individuals with an ASD diagnosis provides a basis for understanding how the pattern of affect displayed across FTF and PAB contexts may be important in distinguishing HR from LR. Unlike LR 6-month-olds, the HR group did not shift their smiling behavior with change in social interaction context. It appears that HR 6-month-olds did not augment their social-emotional behavior to match changes in the social "rules" of the interaction. Individuals with an ASD diagnosis demonstrate difficulty with cognitive and social flexibility (e.g., Hill, 2006; Ozonoff, Strayer, McMahon, & Filloux, 1994), following implicit social "rules" (Klinger, Klinger, & Pohling, 2007), and a lack of social awareness, perception, and response to social cues (e.g., other's facial expressions; Baron-Cohen, 1988). Based on analyses described here (i.e., assessment of group differences in HR and LR 6-month-olds as a whole group), lack of shift in social-emotional behavior with shift in social context appears to be possible characteristic of general HR status at 6 months. However, in the below discussion, it

becomes clear that lack of affective shift by context is potentially unique to HR-ASD 6-month-olds.

***11-month-olds' affect and looking.*** The pattern of risk group differences observed at 11 months differs from the pattern observed at 6 months of age. Consistent with findings at the 6 month age point analyses conducted at the 11 month age point revealed significant risk group differences in smiling and directed smiling. However, the pattern of risk group differences in 11-month-olds' smiling and directed smiling behavior differed from the pattern observed at 6 months. At 6 months, HR infants displayed a high degree of smiling that did not change from FTF to PAB, while LR infants smiled significantly more during PAB than FTF. In addition, HR 6-month-olds directed their smiles more than LR 6-month-olds, but only in the context of FTF. In contrast, HR 11-month-olds smiled and directed smiles more than LR 11-month-olds *in both* FTF and PAB contexts. A final difference between findings at 6 and 11 months was in the area of visual attention. At 6 months HR infants looked toward their mother significantly more than LR infants; however, no significant differences in looking behavior were found at 11 months of age.

***Considering change in affect and looking behavior from 6- to 11-months.*** In comparison to LR 6-month-olds, HR 6-month-olds displayed increased looking to mother, smiling and directed smiling. However, in comparison to LR 11-month-olds, HR 11-month-olds showed increased smiling and directed smiling without increased visual attention to their mother. In addition, at 6-months HR infants displayed a lack of shift in affective behavior across change in interaction context, but by 11-months HR infants displayed a cross-context shift in affect that mirrored LR infants' behavior at 6- and 11-months. Possible explanations for differences in risk group effects by age point are considered below.

HR 6-month-olds increased looking to their mother is described as potentially related to difficulty with visual disengagement that is possibly related to “sticky” attention. It is further postulated that this type of visual attention could lead to high arousal as evidenced by increased smiling during FTF and maintenance of this level of smiling during PAB. At 11 months, HR infants do not display increased looking to mother, but continue to display increased positive affect across contexts in comparison to LR infants. How can HR 11-month-olds’ behavior be explained in a way that is consistent with the idea of sticky attention and high arousal?

It is possible that gross abilities to visually disengage from stimuli are developed by 11 months in the HR sample, but more subtle differences in visual attention still exist. Global coding of looking in an interaction setting does not allow for the detection of more subtle, but meaningful differences in visual attention. If subtle differences in visual processing exist at 11 months, these atypicalities could lead to behavioral evidence of heightened arousal (i.e., increased overall smiling and directed smiling) in HR infants. A second possible explanation for lack of risk group differences in visual attention, but remaining risk group differences in displays of affect is that while global visual abilities may be sufficiently developed in the HR sample by 11 months, the ability to affectively engage in social interaction and/or regulate arousal is not. If at 6 months, the visual perceptual system of the HR infant is not functioning in a manner that allows for visual disengagement – an ability that facilitates the development of typical cycles of mother-infant affective engagement and arousal regulation (e.g., Porges, 1992) – then it may take HR infants much more time to develop affective behavior that mirrors that of LR 11-month-olds.

It is also important to consider why HR and LR infants may demonstrate different patterns of affective response across FTF and PAB contexts at 6 and 11 months of age. HR 6-month-olds displayed consistently high levels of smiling behavior across FTF and PAB contexts,

while LR 6-month-olds, LR 11-month-olds, and HR 11-month-olds showed an increase in smiling from FTF to PAB interaction contexts. It is possible that it takes HR infants more time and social experience to learn the implicit social rules that govern mother-infant FTF interaction and social-action games. If this is the case, it is not surprising that HR infants do not appear to recognize and react to a FTF-PAB shift in social interaction context at 6 months, but do so at 11 months.

*Affect and looking as characteristics of HR-no ASD and HR-ASD infants.* Having considered risk group differences between the full HR and LR sample at 6 and 11 months of age, it is now possible to consider which behaviors measured during the first year of life may uniquely characterize HR-no ASD, HR-ASD, and atypical development in toddlerhood. Two sets of exploratory analyses completed in the current study provide information about how early behaviors may characterize HR-no ASD, or mark later atypical development or ASD diagnosis. These analyses include: a) the comparison of ANOVA results conducted with and without HR-ASD infants, and b) logistic regression analyses predicting diagnostic outcome from early infant behavior.

*Reasons for caution.* For a few reasons, these analyses must be interpreted with caution. First, not all infants included in the 6 and 11 month sample reached diagnostic outcome by the completion of the current study period, and thus, the diagnostic outcome of some infants remained undetermined. Infants who were not seen for diagnostic outcome determination during the current study period could ultimately receive a diagnosis of ASD, or more general atypical development, and the addition of these data could augment conclusions regarding early predictors of ASD and/or atypical development.

A second reason for caution is specific to logistic regression analyses. In the current study, logistic regression analyses were completed with a subset of the total sample – those infants who had completed at least one diagnostic outcome time point during the time period of the present study. Thus, the size of each group included in the logistic regression was quite small, with some groups including less than five cases. It is well known that small sample sizes can be problematic when conducting this type of analysis (e.g., Nemes, Jonasson, Genell, & Steineck, 2009). For this reason, findings from logistic regression analyses predicting diagnostic outcome are not interpreted here. Tentative conclusions are drawn only from interpretation of ANOVA analyses.

*Interpreting ANOVA results.* Comparing results of ANOVA with and without HR-ASD infants allows for tentative conclusions to be made about the affective and looking behaviors of infant siblings that distinguish HR-no ASD from LR infants and may uniquely characterize HR-ASD. Results at the 6 month age point are considered first, followed by results at the 11 month age point.

At 6 months, ANOVA analyses including the full HR and LR sample revealed a significant interaction effect of risk-status and interaction context on the proportion of time infants spent smiling, in directed smiling, and in neutral affect. HR 6-month-olds' affective displays were highly positive and remained consistent across FTF and PAB contexts, while LR 6-month-olds displayed increased positive affect and decreased neutral affect during PAB as compared to FTF. When HR-ASD infants were excluded from these analyses, significant interaction effects disappeared for all affect variables. This suggests that highly positive affect that is unaltered by change in social interaction context is unique to HR-ASD 6-month-olds. Visual examination of the mean amount of smiling, directed smiling, and neutral affect displayed

in these contexts by HR-ASD, HR-no ASD, and LR infants supports this conclusion (see Table 19).

In contrast, HR and LR group differences in looking behavior at 6 months remained after excluding HR-ASD 6-month-olds. Analyses comparing a) the full sample of HR and LR 6-month-olds, and b) HR-no ASD and LR 6-month-olds indicate that, compared to LR 6-month-olds, HR 6-month-olds as a group and HR-no ASD 6-month-olds display increased looking toward mother. Thus, increased looking to mother may define HR status.

At 11 months, the proportion of interaction time spent in smiling and directed smiling differed for HR and LR infants when all infants were included in the analyses. When HR-ASD infants were excluded, differences in the positive affect displayed by HR-no ASD and LR 11-month-olds remained. Thus, at 11 months increased smiling and directed smiling may characterize HR status.

In sum, these exploratory analyses suggest that facial expression production may characterize HR-ASD at 6 months, and that looking behavior at 6 months and emotion production at 11 months may characterize HR status. Two prior infant sibling studies have noted non-significant trends for infants later-diagnosed with ASD to display increased social smiling at 6 months of age (Ozonoff et al., 2010; Rozga et al., 2011). Current study findings are somewhat consistent with past studies, indicating that increased smiling and directed smiling and decreased neutral affect may help define ASD at 6 months. Intriguingly, increased visual attention to mother appears to define HR status at 6 months, as does increased smiling and directed smiling at 11 months. It is suspected that increased looking to mother at 6 months may be the result of difficulty with visual engagement in the HR sample. Difficulty disengaging from mother at 6 months may contribute to increased smiling observed at 11 months.

## **4.2 ASSESSING EMOTION PERCEPTION**

In addition to assessing the social-emotional behavior (i.e., facial expressions and looking) produced by mothers and infants in an interaction setting, the present study examined how HR and LR infants visually attended to facial expression stimuli using eye-tracking methods. Three general conclusions can be drawn regarding infant siblings visual attention to emotion faces. First, smile intensity augments infants' visual attention to smile/neutral face pairs at both 6 and 11 months of age. Second, risk group differences in visual attention to facial expression stimuli were evident at 6 months, but not 11 months of age. HR 6-month-olds looked at stimuli and the internal features of smile and neutral faces more than LR-6-month-olds. Third, the pattern of looking to facial expression stimuli exhibited by HR 6-month-olds distinguished the full sample of HR 6-month-olds and HR-no ASD 6-month-olds from LR infants, which suggests that this pattern of visual attention characterizes general HR status at 6 months. Each of these general conclusions is considered in greater detail below.

### **4.2.1 Consideration of Smile Intensity**

At 6 months of age, the intensity of the smile included in the smile/neutral stimulus pairing augmented both HR and LR infants' looking to a) the smiling versus neutral half of the stimulus, b) the internal features of the face, and c) the eye-mouth looking ratio. More of the infants' visual attention was spent looking to the smile side of the smile/neutral face pair when the smile



was at its' highest intensity. HR and LR 6-month-olds attended to the inner features of the smile/neutral faces more when the smile was a high intensity or low intensity smile than when the smile was prototypical. And finally, 6-month-olds looked more at the eyes than the mouth when the smile was at its lowest intensity, in comparison to when the smile was prototypical or high intensity. These findings depict the 6-month-old infant as visually drawn to highly exaggerated smiles, more interested in the internal features of a face when the smile is extreme (i.e., either high or low intensity), and most interested in the eyes most when a smile is low intensity and the mouth region of the face is a relatively less salient visual cue.

At 11 months, the intensity of the smile included in the smile/neutral pair affected all visual attention variables. For all but one of these measures highly exaggerated smiles appeared to draw the HR and LR infants' visual attention more than prototypical and low intensity smiles. Infants at this age point looked longest at smile/neutral pairings when the pairing included a high intensity smile. HR and LR 11-month-olds also looked for a greater proportion of time at the smile side of the stimuli, the face area, and the internal features of the face when the stimuli included a high intensity smile than when the stimuli included prototypical or low intensity smiles. In addition, 11-month-olds looked more at the eyes than the mouth when stimuli included a low intensity smile, in comparison to when stimuli included a smile that was prototypical or high intensity. These findings depict the 11 month-old infant as visually drawn to highly exaggerated smiles; high intensity smiles appear to orient the 11-month-old infant to the face area, internal features, and the mouth region of the face.

#### **4.2.2 Consideration of Infants' Risk-Status**

Intriguingly, an effect of risk status on infant's visual attention to smile/neutral face pairs was found at 6 months only. Compared to LR 6-month-olds, HR 6-month-olds looked at smile/neutral face pairs for a greater amount of time and looked at the internal features of the face for a greater proportion of total looking time. At the 11 month age point, risk group differences were not evident. That is, HR and LR 11-month-olds visually attended to neutral and affective faces in a similar manner.

It is also possible that the increased visual attention to face pairs represents a difficulty visually disengaging from stimuli for HR 6-month-olds that resolves by 11 months of age. This pattern of looking may indicate “sticky” attention, an attentional style that has distinguished HR from LR infants in prior infant sibling studies of visual attention (e.g., Holmboe, Elsabbagh, Volein, Tucker, Baron-Cohen et al., 2010; Zwaigenbaum et al., 2005), and is consistent with deficits in flexibility and set shifting that defines the cognitive profile of older individuals with an ASD diagnosis (e.g., Hill, 2006; Ozonoff, Strayer, McMahon, & Filloux, 1994). It is also possible that the ability to visually disengage develops later for HR infants than LR infants. Difficulties with visual disengagement, or delayed development in the ability to disengage, may explain risk group differences in visual attention at 6 months and their disappearance by 11 months of age.

#### **4.2.3 Visual Attention Characteristics of HR-no ASD and HR-ASD Infants**

Having considered risk group differences between the full HR and LR sample at 6 and 11 months of age, it is possible to consider which aspects of visual attention measured during the

first year of life may uniquely characterize HR-no ASD, HR-ASD, and atypical development in toddlerhood. Two sets of exploratory analyses conducted as a part of the current study provide information about how early visual attention may characterize HR-no ASD, or mark later atypical development or ASD diagnosis. These analyses include: a) the comparison of ANOVA results conducted with and without HR-ASD infants, and b) logistic regression analyses predicting diagnostic outcome from early infant behavior.

***Reasons for caution.*** These analyses must be interpreted with caution for two reasons. First, not all infants who participated in the assessment of emotion perception at 6 and/or 11 months of age participated in a diagnostic outcome assessment during the time course of the present study. Therefore, the diagnostic outcome of some participants remained undetermined at the conclusion of this study. Infants who were not seen for diagnostic outcome determination during the current study period could ultimately receive a diagnosis of ASD or more general atypical development. The addition of these data could augment conclusions regarding early predictors of ASD and/or atypical development.

A second reason for caution relates to logistic regression analyses specifically. Only a subset of participants, those infants who had completed at least one diagnostic outcome time point during the time period of the present study, could be included in logistic regression. For this reason, the size of each group included in the logistic regression was small, with some groups including less than four cases. Small sample sizes can be problematic when conducting this type of analysis (e.g., Nemes, Jonasson, Genell, & Steineck, 2009). For this reason, findings from logistic regression analyses predicting diagnostic outcome are not interpreted here. Tentative conclusions are drawn only from interpretation of ANOVA analyses.

***Interpreting ANOVA results.*** Analysis of risk group differences between the full sample of HR and LR infants indicated significant risk group differences in visual attention to smile/neutral face pairs at 6 months only. In comparison to LR 6-month-olds, HR 6-month-olds spent more time looking at smile/neutral face pairs and the inner features of the face. When HR-ASD 6-month-olds were excluded from analyses, results were consistent with full sample analyses. HR-no ASD 6-month-olds spent more time looking at smile/neutral face pair stimuli and attended to the internal features of the face more than LR 6-month-olds. Thus, at 6 months, increased looking to stimuli and the inner features of the face may characterize HR status. At 6 months, increased visual attention to smile/neutral face stimuli in the eye-tracking paradigm mirrors increased visual attention toward mother (i.e., an expressive face) during a naturalistic interaction. A lack of difference in visual attention between HR and LR 11-month-olds is also consistent across eye-tracking and face-to-face interaction contexts. Integrative conclusions regarding facial expression perception and production findings are provided below.

#### **4.3 INTEGRATIVE CONCLUSIONS: EMOTION PRODUCTION AND PERCEPTION**

The present study explored emotion production and perception in a group of infants at HR and LR for ASD at 6 and 11 months of age. An overarching goal was to describe not only the quality of these aspects of social-emotional development, but also the timing of their emergence as characteristics that distinguish HR from LR status, and possibly differentiate HR-ASD from HR-no ASD and LR infants. An additional aim of the current study was to suggest how emotion perception and production develop in tandem for HR and LR groups. A final goal was to assess

the influence of context on infant siblings' emotion development. Consideration of multiple contextual variables (e.g., mother-infant interaction type, intensity of smiles viewed) as potentially augmenting the emotional development of infant siblings reflects an attempt to understand infant siblings' emotion development as a transactional process. A discussion that integrates the theoretical background and results of the current study is provided below.

#### **4.3.1 Revisiting Theory**

The findings of the present study are conceptualized within the framework of the experience-expectant model of emotion development (Leppanen & Nelson, 2009) and the transactional model of general child development (Sameroff, 1975, 2009). Leppanen and Nelson (2009) theorize that the neurological and visual systems that process emotion cues are experience-expectant. They argue that neurocognitive systems “expect” input gained through experience with emotion cues, but that the development of these systems are also “dependent” on this experience. Thus, Leppanen and Nelson (2009) suggest that the transactions between what is internal and external to the infant shape neurological and visual systems. An infants' emotion production (i.e., social-emotional behavior) in an interaction and an infants' visual perception of emotion shape one another over time. Sameroff's (1975, 2009) transactional model of development is a more general developmental theory that suggests child development occurs as a result of bidirectional influences. He argues that development itself is a product of continuous transactions that occur over time between an individual and the different aspects of their environment (e.g., the social context).

These theories provide a frame for integrating findings regarding infant siblings' ability to produce and perceive emotion cues, while remaining cognizant of the fact that these systems develop in relation to one another and the external environment. The below discussion integrates findings from paradigms assessing facial expression production and perception to describe how these abilities characterize HR status and are potentially important to the characterization of HR-ASD.

#### **4.3.2 Emotion Production and Perception in HR-no ASD Infants**

Differences in the emotion production and perception of HR-no ASD infants and LR infants indicate behaviors that may characterize HR status. In the context of a mother-infant interaction paradigm and an eye-tracking paradigm, visual attention to faces distinguished HR-no ASD 6-month-olds from LR 6-month-olds. Facial expression production (i.e., smiling, neutral, crying behavior) measured during mother-infant interaction does not distinguish HR-no ASD from LR 6-month-olds. In contrast, facial expression production, but not visual attention distinguished HR-no ASD infant from LR infants at 11 months of age.

During mother-infant interaction HR-no ASD 6-month-olds looked toward their mother significantly more than LR 6-month-olds. At 11 months, smiling and directed smiling, not visual attention, distinguished HR-no ASD infants from LR infants. Based on infant siblings' behavior during a mother-infant interaction, it appears that HR status is characterized by increased looking toward their mother at 6 months, as well as increased smiling and directed smiling at 11 months.

The quality of infant siblings' visual attention to emotion measured using eye-tracking methods was consistent with the quality of looking behavior observed during mother-infant

interaction. In an eye-tracking context, HR-no ASD 6-month-olds displayed increased looking in comparison to LR 6-month-olds. HR-no ASD 6-month-olds looked at smile/neutral face pairs and the internal features of these faces significantly longer than LR 6-month-olds. At 11 months, there were no differences in HR-no ASD and LR infants' looking.

Across two contexts, HR-no ASD infants displayed increased looking to facial expressions of emotion at 6 months, but not at 11 months. At 11 months, affect production distinguished the groups. Although further studies are needed to test the reliability of these findings, the current study suggests that increased looking to emotion-laden faces, whether in an eye-tracking paradigm or naturalistic interaction, characterized HR-no ASD infants at 6 months. At 11 months, visual attention no longer distinguished these groups. Instead, increased smiling and directed smiling during mother-infant interaction uniquely characterized HR-no ASD infants.

Based on these findings, it appears that HR-no ASD infants' visual perception may differ from that of LR infants at 6 months. This quality of emotion development emerges prior to observable differences between HR-no ASD and LR infants. Considering that emotion perception and production abilities develop through bidirectional influence, and that developments in emotion perception influence the trajectory of other aspects of emotion development, it is possible that the quality of HR-no ASD 6-month-olds' visual attention contributes to HR-no ASD 11-month-olds' increased smiling behavior during social interaction. Typically developing infants learn to visually disengage from arousing stimuli, a skill that assists in the development of arousal regulation during face-to-face interaction (e.g., Field, 1981). If HR-no ASD infants have difficulty disengaging from facial expressions of emotion (i.e., arousing stimuli) at 6 months, this difficulty could have cascading, downstream effects on affect

regulation abilities. Increased smiling and directed smiling in HR-no ASD 11-month-olds could reflect such regulation difficulty, and may be related to early differences in visual attention.

#### **4.3.3 Emotion Production and Perception in HR-ASD Infants**

Because of the current study's small sample size, and small number of HR-ASD infants, it is quite challenging to draw conclusions about the behaviors that characterize HR-ASD. Findings from ANOVA analyses of emotion production and perception, conducted with and without HR-ASD infants, suggest behaviors and patterns of behavior during the first year of life that should be further investigated as possible characteristics of HR-ASD in studies with larger sample size. Since the current study's analyses were exploratory, conclusions are tentative.

Behaviors considered possibly important to the definition of HR-ASD are those behaviors that were found to distinguish HR and LR infants in full-sample ANOVA analyses, but did not distinguish HR-no ASD and LR infants once HR-ASD were removed from analyses. Only facial expression production observed during mother-infant interaction at 6 months meet these criteria. Behaviors possibly relevant to the definition of ASD during the first year of life include: smiling, directed smiling, and neutral affect.

Prior to the removal of HR-ASD 6-month-olds from full sample analyses of risk-group differences in affect, a significant interaction of risk-status and interaction context was found for smiling, directed smiling, and neutral affect. HR 6-month-olds as a whole did not shift their amount of smiling, directed smiling and neutral affect based on change in interaction type (i.e., FTF to PAB), whereas LR 6-month-olds augmented their affective displays based on context. In comparison to LR 6-month-olds, HR 6-month-olds displayed more positive and less neutral



affect during FTF interaction and maintained this affective response during PAB. LR infants displayed more positive affective response to PAB than FTF. When HR-ASD 6-month-olds were removed from analyses, the significant interaction between risk-status and interaction context for smiling, directed smiling, and neutral affect was no longer evident. Therefore, the lack of affective augmentation based on change in social-emotional context may occur for HR-ASD infants only at 6 months. HR-ASD 6-month-olds display context-indiscriminant, high positive affect. Scatterplots of individual-level data, shown in Figures 9 through 14, indicate that HR-ASD 6-month-olds are indeed behaving in this manner. It is important to note, as it bolsters this rather tentative finding, that prior infant sibling studies also report a non-significant trend for infants later diagnosed with ASD to display more social smiling at 6 months of age (Ozonoff et al., 2010; Rozga et al., 2011).

These findings demonstrate how important it is to consider contextual influences when attempting to understand the emotional development of infant siblings. Had emotion production been observed in the PAB setting alone, HR and LR infants would appear quite similar to one another. Possible indicators of HR-ASD come to light when looking at the FTF, not PAB, portion of the interaction and when looking across FTF and PAB contexts. Thus, transactional processes that occur between the infant and the environment are important to understanding the different trajectories of emotion development in HR-no ASD, HR-ASD, and LR infants.

#### **4.4 LIMITATIONS**

Throughout the above discussion, study limitations have been noted. The primary limitation of this study is small sample size. Unfortunately, this is not a problem unique to the current study,

but an issue that commonly occurs within infant sibling research (e.g., Zwaigenbaum et al., 2005). In fact, the sample size of the current study is comparable to other published infant sibling research that have assessed similar behavior and utilized similar experimental study procedures (e.g., Cassel et al., 2007; Merin et al., 2007). A clear difference between the current study and previously published studies is in the number of participants who have been diagnosed with ASD. Studies which have attempted to predict ASD diagnosis from behaviors measured during the first year of life have included many more infants with an ASD diagnosis (e.g., 25 later-diagnosed and 25 typically developing participants; Ozonoff et al., 2010). When only three to seven infants at each age point, in each study have received a diagnosis of ASD, as is the case in the current study, description of early characteristics of ASD is exploratory at best.

In the present study, small sample size led to analytical difficulties. In most cases, the logistic regression analyses attempted in the current study did not proceed beyond preliminary analyses of model fit. This is because small sample size made it difficult to accurately fit a prediction model. Unfortunately, there are no statistical prediction methods that are robust to such small sample sizes. In fact, the most common recommendation made to ameliorate this statistical problem is to increase sample size.

The robustness of current and past research investigating infant sibling's emotion development can also be considered a limitation. Present study findings are based on small samples with high variability in behaviors measured. Past studies of emotion production and perception have similar shortcomings. In addition, there are very few prior investigations of infant siblings' emotion production and perception. The limitations of this study, and the group of infant sibling studies that assess emotion development, indicate a need for additional research and replication of findings in larger samples.

## 4.5 FUTURE DIRECTIONS

Future research should be completed with a larger sample. Conducting the current study's interaction and eye-tracking paradigms in a multisite study framework would greatly increase sample size in a relatively short amount of time. The most robust infant sibling findings come from infant sibling research that utilizes a multisite data collection model (e.g., Zwaigenbaum et al., 2005).

Findings from the current study point to two areas for future research. First, it would be informative to measure the physiological arousal of HR and LR infants while they participate in face-to-face interaction and when presented with emotion-laden stimuli. The current study findings suggest that HR infants have difficulty with visual disengagement which may influence affective arousal. This may be evidence of altered stress response or arousal in HR infants. Concrete measures of arousal would indicate whether this explanation for increased positive affect in HR infants is appropriate. A second area for future research includes the direct measurement of visual disengagement in the context of face-to-face interaction. Measuring this behavior would clarify whether infants' increased looking toward their mother, as observed in the present study, is indeed related to difficulty with disengagement. Measurement of saccades toward and away from stimuli could easily be assessed within current study paradigms. These data would help explain findings of increased visual attention as a characteristic of HR status.

## 4.6 CONCLUSIONS

Four summative conclusions can be made from the present study findings. First, HR and LR infants differ in emotion production and perception as early as 6 months of age. Visual attention appears to distinguish HR-no ASD from LR 6-month-olds, while affect production may distinguish HR-ASD infants from HR-no ASD and LR 6-month-olds. Second, *increased* visual attention to emotion-laden faces and *increased* positive affect appear to characterize HR status and potentially HR-ASD. Third, infants' age and qualities of the interaction context appear to augment risk-specific differences in emotion production and perception. Finally, continued infant sibling investigations with larger samples are necessary to understand differences in the emotion development of HR-no ASD, HR-ASD and LR infants.

## 5.0 TABLES

Table 1. Interaction Paradigm – Infant & Mother Characteristics at the 6-month Time Point

	HR (n=26)	LR (n=24)
<i>Infant Characteristics</i>		
<b>Chronological age*</b>		
M (SD)	6.5 (0.5)	6.5 (0.4)
Range	5.8-7.7	6.0-7.1
<b>Verbal DQ*</b>		
M (SD)	85.82 (24.8)	83.23 (13.0)
Range	20-142	58-108
# Missing Cases	2	2
<b>Nonverbal DQ*</b>		
M (SD)	108.43 (25.8)	109.32 (24.8)
Range	58-170	75-175
# Missing Cases	3	1
<b>Gender (%)</b>		
Male	46	50
Female	54	50
<b>Race (%)</b>		
Caucasian	92	100
Hispanic	8	0
African American	0	0
<i>Mother Characteristics</i>		
<b>Education (%)</b>		
High school/some college	42.3	4.2
College degree	23.1	33.3
Graduate/professional degree	34.6	62.5

*Note.* HR: high-risk, LR: low-risk, DQ: developmental quotient; \*Chronological age, verbal DQ, and nonverbal DQ did not differ significantly for HR and LR groups ( $p < 0.05$  for all comparisons).

Table 2. Interaction Paradigm – Infant & Mother Characteristics at the 11-month Time Point

	HR (n=24)	LR (n=33)
<i>Infant Characteristics</i>		
<b>Chronological age*</b>		
M (SD)	11.4 (0.49)	11.4 (0.41)
Range	10.8-12.5	10.4-12.3
<b>Verbal DQ*</b>		
M (SD)	92.79 (14.9)	96.25 (15.3)
Range	55-121	104-155
Missing Cases (n)	1	2
<b>Nonverbal DQ*</b>		
M (SD)	122.17 (18.2)	123.96 (12.2)
Range	91-173	68-123
Missing Cases (n)	1	1
<b>Gender (%)</b>		
Male	50	48
Female	50	52
<b>Race (%)</b>		
Caucasian	96	94
Hispanic	4	3
African American	0	3
<i>Mother Characteristics</i>		
<b>Education (%)</b>		
High school/some college	37.5	12.1
College degree	25.0	30.3
Graduate/professional degree	37.5	57.6

*Note.* HR: high-risk, LR: low-risk, DQ: developmental quotient; Chronological age, verbal DQ, and nonverbal DQ did not differ significantly for HR and LR groups ( $p < 0.05$  for all comparisons).

Table 3. Eye-Tracking Paradigm – Infant Characteristics at 6 Months

	<b>HR</b> ( <i>n</i> =31)	<b>LR</b> ( <i>n</i> =28)
<b>Chronological age*</b>		
M (SD)	6.6 (0.6)	6.5 (0.4)
Range	5.8-8.3	5.4-7.1
<b>Verbal DQ*</b>		
M (SD)	84.37 (28.9)	86.76 (15.2)
Range	20-110	58-125
# Missing Cases	4	3
<b>Nonverbal DQ*</b>		
M (SD)	113.35 (27.4)	113.27 (27.4)
Range	67-190	75-183
# Missing Cases	5	1
<b>Gender (%)</b>		
Male	52	57
Female	48	43
<b>Race (%)</b>		
Caucasian	97	96
Hispanic	3	0
African American	0	4

*Note.* HR: high-risk, LR: low-risk, DQ: developmental quotient; Chronological age, verbal DQ, and nonverbal DQ did not differ significantly for HR and LR groups ( $p < 0.05$  for all comparisons).

Table 4. Eye-Tracking Paradigm – Infant Characteristics at 11 Months

	<b>HR</b> ( <i>n</i> =37)	<b>LR</b> ( <i>n</i> =32)
<b>Chronological age*</b>		
M (SD)	11.5 (0.57)	11.5 (0.37)
Range	10.5-12.7	10.8-12.3
<b>Verbal DQ*</b>		
M (SD)	87.85 (22.81)	94.13 (17.8)
Range	25-123	46-123
Missing Cases ( <i>n</i> )	1	0
<b>Nonverbal DQ*</b>		
M (SD)	120.25 (17.4)	122.23 (15.9)
Range	91-173	77-154
Missing Cases ( <i>n</i> )	1	0
<b>Gender (%)</b>		
Male	62	50
Female	38	50
<b>Race (%)</b>		
Caucasian	94	94
Hispanic	3	3
African American	3	3

*Note.* HR: high-risk, LR: low-risk, DQ: developmental quotient; Chronological age, verbal DQ, and nonverbal DQ did not differ significantly for HR and LR groups ( $p < 0.05$  for all comparisons).



Table 5. Overlap in Infant Sample Across Interaction & Eye-Tracking Paradigm at 6 and 11 months

	6-months		11-months	
	HR	LR	HR	LR
<b>Participation in both paradigms</b>				
Sample size (n)	19	22	23	28
Proportion of overall sample (%)	73	92	96	85

*Note.* HR: high-risk, LR: low-risk

Table 6. Interaction Paradigm – Diagnostic Classification and Time Point of Diagnosis for 6-month-olds

	<b>HR</b> ( <i>n</i> =24)		<b>LR</b> ( <i>n</i> =20)	
	<i>n</i>	%	<i>n</i>	%
<b>Diagnostic Classification</b>				
ASD	5	20.8	0	0
Language Delay	0	0	0	0
Global Developmental Delay	1	4.2	0	0
Social Concerns	2	8.3	1	5.0
Typically Developing	16	66.7	19	95.0
<b>Met Multiple Classification Criteria</b>	6	25	0	0
<b>Total Typically Developing</b>	16	66.7	19	95.0
<b>Total Atypically Developing</b>	8	33.3	1	5.0
<b>Classification Time Point</b>				
24-months	6	25.0	1	5.0
36-months	8	33.3	2	10.0
48-months	10	41.7	17	85.0

*Note.* HR: high-risk, LR: low-risk, ASD: autism spectrum disorder; Infants could meet multiple classification criteria.

Table 7. Interaction Paradigm – Diagnostic Classification and Time Point of Diagnosis for 11-month-olds

	<b>HR</b> ( <i>n</i> =23)		<b>LR</b> ( <i>n</i> =26)	
	<i>n</i>	%	<i>n</i>	%
<b>Diagnostic Classification</b>				
ASD	4	17.4	0	0
Language Delay	0	0	2	7.7
Global Developmental Delay	2	8.7	0	0
Social Concerns	2	8.7	1	3.8
Typically Developing	15	65.2	23	88.5
<b>Met Multiple Classification Criteria</b>	6	26.1	0	0
<b>Total Typically Developing</b>	15	65.2	23	88.5
<b>Total Atypically Developing</b>	8	34.8	3	11.5
<b>Classification Time Point</b>				
24-months	4	17.4	1	3.8
36-months	8	34.8	3	11.5
48-months	11	47.8	22	84.6

*Note.* HR: high-risk, LR: low-risk, ASD: autism spectrum disorder; Infants could meet multiple classification criteria.

Table 8. Eye-Tracking Paradigm – Diagnostic Classification and Time Point of Diagnosis for 6-month-olds

	<b>HR</b> ( <i>n</i> =20)		<b>LR</b> ( <i>n</i> =21)	
	<i>n</i>	%	<i>n</i>	%
<b>Diagnostic Classification</b>				
ASD	3	15.0	0	0
Language Delay	0	0	1	4.8
Global Developmental Delay	1	5.0	0	0
Social Concerns	3	15.0	1	4.8
Typically Developing	13	65.0	19	90.5
<b>Met Multiple Classification Criteria</b>	5	25.0	0	0
<b>Total Atypically Developing</b>	7	35.0	2	9.5
<b>Total Typically Developing</b>	13	65.0	19	90.5
<b>Classification Time Point</b>				
24-months	6	30.0	2	9.5
36-months	5	25.0	2	9.5
48-months	9	45.0	17	81.0

*Note.* HR: high-risk, LR: low-risk, ASD: autism spectrum disorder; Infants could meet multiple classification criteria.

Table 9. Eye-Tracking Paradigm – Diagnostic Classification and Time Point of Diagnosis for 11-month-olds

	<b>HR</b> <b>(n=35)</b>		<b>LR</b> <b>(n=26)</b>	
	<i>n</i>	%	<i>n</i>	%
<b>Diagnostic Classification</b>				
ASD	7	20.0	0	0
Language Delay	0	0	1	3.8
Global Developmental Delay	0	0	0	0
Social Concerns	5	14.3	2	7.7
Typically Developing	23	65.7	23	88.5
<b>Met Multiple Classification Criteria</b>	9	25.7	0	0
<b>Total Atypically Developing</b>	12	34.3	3	11.5
<b>Total Typically Developing</b>	23	65.7	23	88.5
<b>Classification Time Point</b>				
24-months	10	28.6	2	7.7
36-months	11	31.4	2	7.7
48-months	14	40.0	22	84.6

*Note.* HR: high-risk, LR: low-risk, ASD: autism spectrum disorder; Infants could meet multiple classification criteria.

Table 10. Diagnostic Classification Criteria for Infant Siblings at 24, 36, and 48 Months

	Criteria 1 – Testing Results	Criteria 2 – Clinical Review	Criteria 3 – Supplemental Information
<b>ASD</b>	Meets <u>at least</u> spectrum cutoffs of all three diagnostic totals: Communication Total, Social Interaction Total, and Communication + Social Interaction Total	Clinical review by clinical psychologist is required to warrant this outcome	
<b>Social Concerns</b>	One or both of the following: <ul style="list-style-type: none"> <li>• Meets at least spectrum cutoffs on the Social Interaction total <b>ONLY</b> (i.e., 4 points or more)</li> <li>• Communication + Social Interaction Total within 2 points (or less) of spectrum cutoffs</li> </ul>	Clinical psychologist may place a child meeting spectrum cutoffs here, in the case that a diagnosis of ASD is not appropriate. Additionally, <u>all</u> infants in this outcome must be reviewed by clinical psychologist to determine the cause for social concerns (i.e., Criteria 3) or if exclusion is necessary	Reasons that Criteria 1 may be displayed <ul style="list-style-type: none"> <li>• Shyness and/or anxiety</li> <li>• Behavioral issues</li> <li>• Due to Language Delay</li> <li>• ASD-like</li> </ul>
<b>Global Developmental Delay</b>	Visual Reception <u>and</u> Receptive Language Mullen scores fall at least 1.5 SD below the normative mean. Other domains of the Mullen may or may not also fall 1.5 SD below the mean	Clinical psychologist can exclude any child based on clinical opinion, but inclusion is dependent on concerning Mullen scores (however clinical opinion may place infants in this outcome even if scores do not quite meet the 1.5 SD cutoff)	
<b>Language Delay</b>	One of the following:	Clinical psychologist may include or exclude any child based on	This outcome could be a delay in:

	<ul style="list-style-type: none"> <li>• Mullen scores fall at least 1.5 SD below the normative mean for Expressive and/or Receptive Language <u>ONLY</u></li> <li>• If Words Produced falls at or below the 10<sup>th</sup> percentile, it may warrant this outcome – Clinical Review Required</li> </ul>	clinical opinion, although issues surrounding articulation will not be included	<ul style="list-style-type: none"> <li>• Expressive Language</li> <li>• Receptive Language</li> <li>• Both Expressive and Receptive Language</li> </ul>
<b>Typically Developing</b>	Child must not meet any of the criteria listed above (however, they may have deficits in Gross Motor, Fine Motor, and/or Visual Reception Mullen scores)	Any children with invalid testing results may be included here by staff clinical psychologist	

Table 11. Interaction Paradigm - Range and Mean Duration of FTF and PAB Episodes by Risk-Status and Age

	HR	LR
<i>6-months</i>		
<b>FTF</b>		
Mean (SD)	118.96 (12.8)	121.56 (10.9)
Range	76-154	78-144
<b>PAB</b>		
Mean (SD)	59.78 (3.9)	59.70 (3.5)
Range	52-72	53-70
<i>11-months</i>		
<b>FTF</b>		
Mean (SD)	113.92 (18.8)	112.77 (20.0)
Range	62-127	49-128
<b>PAB</b>		
Mean (SD)	60.28 (3.6)	58.39 (7.8)
Range	48-67	21-69

*Note.* HR: high-risk, LR: low-risk, FTF: face-to-face, PAB: peek-a-boo; Mean duration of FTF and PAB did not differ for HR and LR groups at 6 or 11 month age points.



Table 12. Interaction Paradigm - Operational Definition of Infant FACS and Looking Dependent Variables

<b>Dependent Variable</b>	<b>Operational Definition</b>
<i>Smiling</i>	The numerator of this percent duration variable was defined as the time spent in any FACS code that included an AU12 (i.e., the upturning of the corner of the mouth): AU 12, AU 12+6, AU 12+25, AU 12+25+6, AU 12+26/27, AU 12+26/27+6. These variations in smiling codes reflect the degree of intensity of a smile. For the purposes of these analyses, the amounts of time spent in each smiling intensity code were combined to determine “overall smiling”. The denominator of this percent duration variable was calculated by subtracting the amount of time coded as “Cannot See Infant FACS” from the total interaction time. Thus, the denominator represented the total amount of interaction time where a FACS code (e.g., smiling code, neutral code, cry face code) could be assigned.
<i>Directed Smiling</i>	The numerator of this percent duration variable was defined as time spent in any FACS code that included an AU 12 (as described above), <i>while “looking at their mother” was also occurring</i> . The denominator of this percent duration variable was defined by subtracting a) the amount of time coded as “Cannot See Infant FACS” and “Cannot See Infant Looking” simultaneously, b) the total additional time coded as “Cannot See Infant FACS” alone, and c) the total additional time coded as “Cannot See Infant Looking” alone. Thus, the denominator represented the total amount of interaction time where a smiling FACS code <i>and</i> looking to mother could possibly occur at the same time.
<i>Crying</i>	The numerator of this percent duration variable was defined as time spent in the FACS AU combination AU 20+4. The facial muscles in this combination create what is known as a “cry face” (Cassel et al., 2007), which is defined by the lateral stretching of the lips (AU20) and lowering of the brow (AU4). The denominator for this variable was calculated by subtracting the amount of time coded as “Cannot See Infant FACS” from total interaction time. Thus, the denominator represented the total amount of interaction time where a FACS code (e.g., smiling code, neutral code, cry face code) could be assigned.
<i>Neutral Affect</i>	The numerator of this percent duration variable was defined as time spent in any FACS code other than those that defined “Smiling” and those that defined “Crying”. The denominator of this variable was calculated by

	subtracting the amount of time coded as “Cannot See Infant FACS” from total interaction time. Thus, the denominator represented the total amount of interaction time where a FACS code (e.g., smiling code, neutral code, cry face code) could be assigned.
<i>Looking at Mother</i>	The numerator of this percent duration variable was defined as time spent in the code “looking to mother’s body”. The denominator of this percent duration variable was calculated by subtracting the code “Cannot See Baby Looking” from the total interaction duration.
<i>Looking Away</i>	The numerator of this percent duration variable was defined as time spent in the code “looking away from mother”. The denominator of this percent duration variable was calculated by subtracting the code “Cannot See Baby Looking” from the total interaction time.

Table 13. Interaction Paradigm - Reliability Scores between Primary and Secondary Coders of Infant FACS and Looking Variables

	Primary Coder-Secondary Coder 1		Primary Coder-Secondary Coder 2	
	FACS Codes	Looking Codes	FACS Codes	Looking Codes
<i>6-months</i>				
<b>FTF</b>				
Mean Percent Agreement	76	81	78	85
Cohen's Kappa	0.71	0.72	0.74	0.78
<b>PAB</b>				
Mean Percent Agreement	76	80	81	88
Cohen's Kappa	0.71	0.70	0.77	0.81
<i>11-months</i>				
<b>FTF</b>				
Mean Percent Agreement	80	83	84	90
Cohen's Kappa	0.75	0.73	0.81	0.84
<b>PAB</b>				
Mean Percent Agreement	76	88	76	90
Cohen's Kappa	0.71	0.80	0.71	0.83

*Note.* FTF: face-to-face, PAB: peek-a-boo, FACS: Facial Action Coding System

Table 14. Interaction Paradigm - Description of Ratings for Mother's Affect

<b>Rating</b>	<b>Description</b>
0	A rating of "0" indicates the mother's face, and therefore her affect, could not be seen by the coder. This occurred when the mother turned out of the camera's view or if she covered her face with her hands, which occurred frequently in PAB.
1	A rating of "1" indicates the mother displayed neutral affect for the majority of the 5 second coding interval. Neutral affect is defined by facial expression only. Neutral affect is the absence of behaviors coded under the rating of "2" and "3". In other words, neutral affect is the absence of positive facial affect indicators, including the upturning of the corners of the mouth.
2	A rating of "2" indicates positive affect displayed by the mother for the majority of the 5 second coding interval. Positive affect under this rating is defined as "low" to "moderate" positive affect. This rating is based solely on facial expression. Facial indicators of positive affect include: the upturning of the lip corners with or without slight opening of the mouth and visibility of the teeth.
3	A rating of "3" indicates high intensity positive affect displayed by the mother for the majority of the 5 second coding interval. High positive affect under this rating is defined by facial expression only. Facial indicators of high intensity positive affect include: the upturning of the lip corners with clear mouth opening, teeth visibility, space between upper and lower teeth, jaw dropping, raised eyebrows, and widening of the eye aperture.

Table 15. Interaction Paradigm - Descriptive Data at 6 months for Each Dependent Measure by Risk-Group

	<b>HR</b> (n=26)		<b>LR</b> (n=24)	
	FTF	PAB	FTF	PAB
<b>Proportion Smiling</b>				
Mean (SD)	0.46 (0.28)	0.56 (0.26)	0.36 (0.26)	0.61 (0.26)
Range	0.0-0.85	0.04-0.94	0.03-0.94	0.01-0.92
<b>Proportion Directed Smiling</b>				
Mean (SD)	0.32 (0.22)	0.49 (0.26)	0.19 (0.17)	0.51 (0.26)
Range	0.0-0.72	0.01-0.89	0.03-0.59	0.0-0.89
<b>Proportion Neutral</b>				
Mean (SD)	0.48 (0.24)	0.42 (0.25)	0.62 (0.26)	0.39 (0.25)
Range	0.15-0.99	0.06-0.96	0.03-0.95	0.08-0.92
<b>Proportion Crying</b>				
Mean (SD)	0.02 (0.08)	0.02 (0.05)	0.01 (0.03)	0.01 (0.02)
Range	0.0-0.42	0.0-0.18	0.0-0.1	0.0-0.1
<b>Proportion Look Adult</b>				
Mean (SD)	0.49 (0.27)	0.76 (0.20)	0.30 (0.18)	0.67 (0.23)
Range	0.04-0.95	0.31-1.0	0.04-0.75	0.09-0.98
<b>Proportion Look Away</b>				
Mean (SD)	0.36 (0.23)	0.20 (0.18)	0.48 (0.23)	0.26 (0.05)
Range	0.05-0.77	0.0-0.60	0.14-0.88	0.0-0.89

*Note.* HR: high-risk, LR: low-risk, FTF: face-to-face interaction, PAB: peek-a-boo

Table 16. Interaction Paradigm - Descriptive Data at 11 months for Each Dependent Measure by Risk-Group

	<b>HR</b> ( <i>n</i> =24)		<b>LR</b> ( <i>n</i> =33)	
	FTF	PAB	FTF	PAB
<b>Proportion Smiling</b>				
Mean (SD)	0.40 (0.25)	0.67 (0.23)	0.26 (0.19)	0.59 (0.25)
Range	0.01-0.86	0.20-0.99	0.0-0.69	0.01-0.98
<b>Proportion Directed Smiling</b>				
Mean (SD)	0.25 (0.21)	0.63 (0.27)	0.16 (0.11)	0.49 (0.24)
Range	0.01-0.73	0.19-1.0	0.0-0.39	0.0-0.98
<b>Proportion Neutral</b>				
Mean (SD)	0.47 (0.20)	0.29 (0.20)	0.57 (0.18)	0.32 (0.20)
Range	0.14-0.83	0.01-0.69	0.25-0.96	0.02-0.83
<b>Proportion Crying</b>				
Mean (SD)	0.09 (0.16)	0.04 (0.08)	0.12 (0.20)	0.08 (0.15)
Range	0.0-0.66	0.0-0.27	0.0-0.75	0.0-0.64
<b>Proportion Look Adult</b>				
Mean (SD)	0.44 (0.22)	0.77 (0.18)	0.36 (0.14)	0.71 (0.20)
Range	0.7-0.84	0.41-0.99	0.11-0.65	0.23-1.0
<b>Proportion Look Away</b>				
Mean (SD)	0.44 (0.18)	0.22 (0.17)	0.51 (0.14)	0.26 (0.19)
Range	0.15-0.85	0.0-0.56	0.17-0.78	0.0-0.77

*Note.* HR: high-risk, LR: low-risk, FTF: face-to-face interaction, PAB: peek-a-boo

Table 17. Interaction Paradigm – Statistical Results for Analysis of Variance at 6 months

	Interaction Context	Risk-Status	Interaction Context x Risk-Status
<b>Smiling</b>			
Full Sample	$F(1,48) = 27.07$ $p < 0.001^{**}$	$F(1, 48) = 0.08$ $p = 0.78$	$F(1, 48) = 4.01$ $p = 0.051^*$
HR-ASD Excluded Sample	$F(1, 43) = 46.78$ $p < 0.001^{**}$	$F(1, 43) = 0.04$ $p = 0.85$	$F(1, 43) = 0.84$ $p = 0.37$
<b>Directed Smiling</b>			
Full Sample	$F(1, 48) = 64.03$ $p < 0.001^{**}$	$F(1, 48) = 1.24$ $p = 0.27$	$F(1, 48) = 5.54$ $p = 0.02^*$
HR-ASD Excluded Sample	$F(1, 43) = 102.99$ $p < 0.001^{**}$	$F(1, 43) = 1.22$ $p = 0.28$	$F(1, 43) = 1.86$ $p = 0.18$
<b>Crying</b>			
Full Sample	$F(1, 48) = 0.05$ $p = 0.82$	$F(1, 48) = 0.36$ $p = 0.55$	$F(1, 48) = 0.00$ $p = 0.99$
HR-ASD Excluded Sample	--	--	--
<b>Neutral</b>			
Full Sample	$F(1, 48) = 21.91$ $p < 0.001^{**}$	$F(1, 48) = 0.45$ $p = 0.51$	$F(1, 48) = 7.36$ $p = 0.01^*$
HR-ASD Excluded Sample	$F(1, 43) = 40.56$ $p < 0.001^{**}$	$F(1, 43) = 0.41$ $p = 0.52$	$F(1, 43) = 2.85$ $p = 0.10$
<b>Looking to Mother</b>			
Full Sample	$F(1, 48) = 155.36$	$F(1, 48) = 6.10$	$F(1, 48) = 1.99$

	$p < 0.001^{**}$	$p = 0.02^*$	$p = 0.16$
HR-ASD Excluded Sample	$F(1, 43) = 197.38$	$F(1, 43) = 7.02$	$F(1, 43) = 0.26$
	$p < 0.001^{**}$	$p = 0.01^*$	$p = 0.61$
<b>Looking Away</b>			
Full Sample	$F(1, 48) = 41.14$	$F(1, 48) = 2.91$	$F(1, 48) = 0.50$
	$p < 0.001^{**}$	$p = 0.09$	$p = 0.48$
HR-ASD Excluded Sample	--	--	--

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*Note.* \*\* indicates significance at  $p < 0.001$ , \* indicates significance at  $p \leq 0.05$



Table 18. Interaction Paradigm – Statistical Results for Analysis of Variance at 11 months

	Interaction Context	Risk-Status	Interaction Context x Risk-Status
<b>Smiling</b>			
Full Sample	$F(1, 55) = 70.35$ $p > 0.001^{**}$	$F(1, 55) = 5.26$ $p = 0.03^*$	$F(1, 55) = 0.60$ $p = 0.44$
HR-ASD Excluded Sample	$F(1, 51) = 64.04$ $p < 0.001^{**}$	$F(1, 51) = 3.91$ $p = 0.05^*$	$F(1, 55) = 0.50$ $p = 0.48$
<b>Directed Smiling</b>			
Full Sample	$F(1, 55) = 100.30$ $p < 0.001^{**}$	$F(1, 55) = 6.08$ $p = 0.02^*$	$F(1, 55) = 0.05$ $p = 0.82$
HR-ASD Excluded Sample	$F(1, 51) = 84.32$ $p < 0.001^{**}$	$F(1, 51) = 5.45$ $p = 0.02^*$	$F(1, 51) = 0.03$ $p = 0.86$
<b>Crying</b>			
Full Sample	$F(1, 55) = 7.00$ $p = 0.01^*$	$F(1, 55) = 1.41$ $p = 0.24$	$F(1, 55) = 0.33$ $p = 0.57$
HR-ASD Excluded Sample	--	--	--
<b>Neutral</b>			
Full Sample	$F(1, 55) = 74.97$ $p < 0.001^{**}$	$F(1, 55) = 2.29$ $p = 0.14$	$F(1, 55) = 1.01$ $p = 0.32$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Mother</b>			
Full Sample	$F(1, 55) = 186.53$ $p < 0.001^{**}$	$F(1, 55) = 2.26$ $p = 0.14$	$F(1, 55) = 0.09$ $p = 0.77$
HR-ASD Excluded Sample	--	--	--

**Looking Away**

Full Sample	$F(1, 55) = 92.86$ $p < 0.001^{**}$	$F(1, 55) = 1.95$ $p = 0.17$	$F(1, 55) = 0.05$ $p = 0.82$
HR-ASD Excluded Sample	--	--	--

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*Note.* \*\* indicates significance at  $p < 0.001$ , \* indicates significance at  $p \leq 0.05$

Table 19. Interaction Paradigm – Descriptive Data for 6-month-olds Characterized as LR, HR-no ASD, and HR-ASD

		LR ( <i>n</i> = 25)		HR-no ASD ( <i>n</i> = 21)		HR-ASD ( <i>n</i> = 5)	
		FTF %	PAB %	FTF %	PAB %	FTF %	PAB %
<b>Smiling</b>	<i>M</i> (SD)	0.35 (0.25)	0.59 (0.26)	0.42 (0.28)	0.59 (0.27)	0.64 (0.24)	0.40 (0.13)
<b>Directed Smiling</b>	<i>M</i> (SD)	0.19 (0.17)	0.50 (0.26)	0.29 (0.19)	0.54 (0.27)	0.46 (0.29)	0.30 (0.06)
<b>Neutral Affect</b>	<i>M</i> (SD)	0.62 (0.25)	0.40 (0.25)	0.51 (0.24)	0.51 (0.24)	0.34 (0.23)	0.60 (0.13)

*Note.* LR: low-risk, HR: high-risk, ASD: autism spectrum disorder, FTF: face-to-face interaction, PAB: peek-a-boo

Table 20. Interaction Paradigm – Diagnostic Classifications Utilized in Logistic Regression for the 6-month Age Point

	<b>HR</b> ( <i>n</i> = 24)	<b>LR</b> ( <i>n</i> = 20)
<b>Diagnostic Classification</b>		
ASD ( <i>n</i> )	5	0
Social Concerns ( <i>n</i> )	2	1
Global Developmental Delay ( <i>n</i> )	1	0
Language Delay ( <i>n</i> )	0	0
<b>Total Classified as Atypically Developing (<i>n</i>)</b>	8	1
<b>Total Classified as Typically Developing (<i>n</i>)</b>	16	19

*Note.* ASD: autism spectrum disorder, HR: high-risk infants, LR: low-risk infants

Table 21. Interaction Paradigm – Diagnostic Classifications Utilized in Logistic Regression at the 11-month Age Point

	<b>HR</b> ( <i>n</i> = 23)	<b>LR</b> ( <i>n</i> = 26)
<b>Diagnostic Classification</b>		
ASD ( <i>n</i> )	3	0
Social Concerns ( <i>n</i> )	3	2
Global Developmental Delay ( <i>n</i> )	2	0
Language Delay ( <i>n</i> )	0	1
<b>Total Classified as Atypically Developing (<i>n</i>)</b>	8	3
<b>Total Classified as Typically Developing (<i>n</i>)</b>	15	23

*Note.* ASD: autism spectrum disorder, HR: high-risk, LR: low-risk

Table 22. Interaction Paradigm – Predicting Atypical Development & ASD from 6 Months: Logistic Regression Results

	Regression Predicting Atypical Development	Regression Predicting ASD
<b>Independent Variables</b>		
<i><b>risk</b></i>		
log-odds ( <i>p</i> -value)	-1.87 ( <i>p</i> = 0.12)	-19.23 ( <i>p</i> = 0.99)
OR (95% CI)	0.16	0.00
<i><b>neutral</b></i>		
log-odds ( <i>p</i> -value)	4.16 ( <i>p</i> = 0.46)	0.55 ( <i>p</i> = 0.94)
OR (95% CI)	63.96 (0.00-4361393.81)	1.72 (0.00-3694563.52)
<i><b>compsmiling</b></i>		
log-odds ( <i>p</i> -value)	7.45 ( <i>p</i> = 0.22)	4.39 ( <i>p</i> = 0.56)
OR (95% CI)	1724.64 (0.01-274465297.60)	80.83 (0.00-202468175.40)
<b>Sample size</b>		
Atypical Development ( <i>n</i> )	9	-
Typical Development ( <i>n</i> )	35	-
ASD Classification ( <i>n</i> )	-	5
Non-ASD Classification ( <i>n</i> )	-	39

*Note.* ASD: autism spectrum disorder, OR: odds ratio, CI: Confidence-interval

Table 23. Interaction Paradigm – Pearson Correlations for Smiling Variables at 11 months

	Overall Smiling (FTF)	Overall Smiling (PAB)	Directed Smiling (FTF)
<b>Overall Smiling (FTF)</b>			
Pearson Correlation	1	0.37	0.65
<i>p</i> -value	-	0.01	0.00
<b>Overall Smiling (PAB)</b>			
Pearson Correlation	0.37	1	0.38
<i>p</i> -value	0.01	-	0.01
<b>Directed Smiling (FTF)</b>			
Pearson Correlation	0.65	0.38	1
<i>p</i> -value	0.00	0.01	-
<b>Directed Smiling (PAB)</b>			
Pearson Correlation	0.29	0.89	0.43
<i>p</i> -value	0.05	0.00	0.00

*Note.* FTF: face-to-face interaction, PAB: peek-a-boo

Table 24. Interaction Paradigm – Predicting ASD from 11 Months: Logistic Regression Results

Regression Predicting ASD	
Independent Variables	
<i>risk</i>	
log-odds ( <i>p</i> -value)	-19.74 ( <i>p</i> = 0.99)
OR (95% CI)	0.00
Sample size	
ASD Classification ( <i>n</i> )	4
Non-ASD Classification ( <i>n</i> )	45
<i>Note.</i> ASD: autism spectrum disorder, OR: odds ratio, CI: Confidence-interval	



Table 25. Eye-Tracking Paradigm – Descriptive Data at 6-months for Each Dependent Measure by Risk-Group and Smile Intensity

	HR (n=31)	LR (n=28)
<b>Total Stimulus Looking</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	17.93 (4.97)	14.15 (7.04)
Range	5.54-28.05	2.13-28.92
<i>Mid-Intensity Smile</i>		
Mean (SD)	18.04 (6.57)	13.48 (8.06)
Range	5.42-29.22	2.82-28.46
<i>High-Intensity Smile</i>		
Mean (SD)	18.06 (5.18)	13.74 (7.02)
Range	7.26-28.16	0.93-28.46
<i>Over All Smile Intensities</i>		
Mean (SD)	54.03 (14.98)	41.37 (20.53)
Range	18.22-79.59	5.88-85.90
<b>Proportion Looking to Smile Half</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.49 (0.10)	0.48 (0.12)
Range	0.32-0.73	0.27-0.77
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.51 (0.11)	0.52 (0.13)
Range	0.31-0.91	0.28-0.78
<i>High-Intensity Smile</i>		
Mean (SD)	0.58 (0.12)	0.55 (0.11)
Range	0.35-0.80	0.29-0.77
<i>Over All Smile Intensities</i>		
Mean (SD)	0.52 (0.07)	0.52 (0.07)
Range	0.40-0.69	0.38-0.69
<b>Proportion Looking to Face</b>		

<i>Low-Intensity Smile</i>		
Mean (SD)	0.76 (0.19)	0.73 (0.18)
Range	0.07-0.92	0.20-0.91
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.75 (0.19)	0.78 (0.14)
Range	0.14-0.75	0.42-0.96
<i>High-Intensity Smile</i>		
Mean (SD)	0.78 (0.20)	0.72 (0.23)
Range	0.11-0.93	0.00-0.92
<i>Over All Smile Intensities</i>		
Mean (SD)	0.76 (0.19)	0.74 (0.16)
Range	0.11-0.92	0.30-0.92
<b>Proportion Looking to Internal</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.66 (0.16)	0.62 (0.15)
Range	0.32-0.93	0.33-0.90
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.62 (0.14)	0.53 (0.18)
Range	0.18-0.82	0.17-0.92
<i>High-Intensity Smile</i>		
Mean (SD)	0.73 (0.17)	0.86 (0.20)
Range	0.30-1.00	0.00-0.86
<i>Over All Smile Intensities</i>		
Mean (SD)	0.67 (0.13)	0.59 (0.12)
Range	0.38-0.87	0.35-0.80
<b>Proportion Eye/Eye+Mouth Looking</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.72 (0.32)	0.77 (0.23)
Range	0.02-1.00	0.32-1.00
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.60 (0.35)	0.52 (0.40)
Range	0.00-1.00	0.00-1.00

*High-Intensity Smile*

Mean (SD)

0.60 (0.36)

0.47 (0.38)

Range

0.00-1.00

0.00-1.00

*Over All Smile Intensities*

Mean (SD)

0.63 (0.32)

0.62 (0.29)

Range

0.01-1.00

0.08-1.00

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*Note.* HR: high-risk, LR: low-risk

Table 26. Eye-Tracking Paradigm – Descriptive Data at 11-months for Each Dependent Measure by Risk-Group and Smile Intensity

	<b>HR</b> ( <i>n</i> =37)	<b>LR</b> ( <i>n</i> =32)
<b>Total Stimulus Looking</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	14.20 (5.07)	13.10 (5.74)
Range	0.84-26.76	2.16-26.66
<i>Mid-Intensity Smile</i>		
Mean (SD)	14.08 (4.82)	12.90 (4.98)
Range	4.21-21.51	3.45-24.71
<i>High-Intensity Smile</i>		
Mean (SD)	15.78 (5.86)	14.43 (5.81)
Range	2.28-27.46	2.24-27.19
<i>Over All Smile Intensities</i>		
Mean (SD)	44.06 (13.30)	40.44 (14.50)
Range	15.22-67.46	10.75-78.56
<b>Proportion Looking to Smile Half</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.52 (0.10)	0.50 (0.10)
Range	0.29-0.78	0.21-0.67
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.53 (0.06)	0.53 (0.13)
Range	0.42-0.68	0.31-0.94
<i>High-Intensity Smile</i>		
Mean (SD)	0.57 (0.07)	0.57 (0.13)
Range	0.41-0.74	0.35-0.84
<i>Over All Smile Intensities</i>		
Mean (SD)	0.54 (0.06)	0.53 (0.06)
Range	0.45-0.70	0.42-0.66
<b>Proportion Looking to Face</b>		

<i>Low-Intensity Smile</i>		
Mean (SD)	0.74 (0.17)	0.76 (0.15)
Range	0.06-1.00	0.36-0.93
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.75 (0.16)	0.74 (0.16)
Range	0.17-0.90	0.46-0.94
<i>High-Intensity Smile</i>		
Mean (SD)	0.79 (0.16)	0.79 (0.15)
Range	0.18-0.95	0.38-0.96
<i>Over All Smile Intensities</i>		
Mean (SD)	0.77 (0.15)	0.77 (0.14)
Range	0.13-0.89	0.39-0.91
<b>Proportion Looking to Internal</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.52 (0.19)	0.58 (0.20)
Range	0.02-0.81	0.19-0.89
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.54 (0.12)	0.51 (0.14)
Range	0.24-0.80	0.28-0.73
<i>High-Intensity Smile</i>		
Mean (SD)	0.62 (0.14)	0.59 (0.23)
Range	0.36-0.86	0.11-0.95
<i>Over All Smile Intensities</i>		
Mean (SD)	0.57 (0.11)	0.56 (0.15)
Range	0.34-0.79	0.26-0.82
<b>Proportion Eye/Eye+Mouth Looking</b>		
<i>Low-Intensity Smile</i>		
Mean (SD)	0.44 (0.35)	0.41 (0.39)
Range	0.00-1.00	0.00-1.00
<i>Mid-Intensity Smile</i>		
Mean (SD)	0.39 (0.034)	0.41 (0.36)
Range	0.00-1.00	0.00-1.00

*High-Intensity Smile*

Mean (SD)

0.34 (0.26)

0.35 (0.37)

Range

0.00-1.00

0.00-1.00

*Over All Smile Intensities*

Mean (SD)

0.37 (0.28)

0.39 (0.34)

Range

0.00-1.00

0.00-0.95

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*Note.* HR: high-risk, LR: low-risk

Table 27. Eye-Tracking Paradigm – Statistical Results for Analysis of Variance at 6 months

	Smile Intensity	Risk-Status	Smile Intensity x Risk-Status
<b>Total Stimulus Looking</b>			
Full Sample	$F(2, 56) = 0.10$ $p = 0.91$	$F(1, 57) = 7.42$ $p = 0.01^*$	$F(2, 56) = 0.22$ $p = 0.80$
HR-ASD Excluded Sample	$F(2, 53) = 0.25$ $p = 0.78$	$F(1, 54) = 6.79$ $p = 0.01^*$	$F(2, 53) = 0.15$ $p = 0.87$
<b>Looking to Smile Half</b>			
Full Sample	$F(2, 56) = 7.14$ $p = 0.002^*$	$F(1, 57) = 0.17$ $p = 0.69$	$F(2, 56) = 0.57$ $p = 0.57$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Face</b>			
Full Sample	$F(2, 56) = 1.06$ $p = 0.36$	$F(1, 57) = 0.16$ $p = 0.69$	$F(2, 56) = 2.74$ $p = 0.07$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Internal</b>			
Full Sample	$F(2, 56) = 6.66$ $p = 0.003^*$	$F(1, 57) = 7.20$ $p = 0.01^*$	$F(2, 56) = 2.16$ $p = 0.13$
HR-ASD Excluded Sample	$F(2, 53) = 0.50$ $p = 0.61$	$F(1, 54) = 7.11$ $p = 0.01^*$	$F(2, 53) = 0.58$ $p = 0.56$
<b>Eye/Eye+Mouth Looking</b>			
Full Sample	$F(2, 56) = 15.59$	$F(1, 57) = 0.48$	$F(2, 56) = 2.56$

	$p < 0.001^{**}$	$p = 0.49$	$p = 0.09$
HR-ASD Excluded Sample	--	--	--

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*Note.* \*\* indicates significance at  $p < 0.001$ , \* indicates significance at  $p \leq 0.05$



Table 28. Eye-Tracking Paradigm – Statistical Results for Analysis of Variance at 11 months

	Smile Intensity	Risk-Status	Smile Intensity x Risk-Status
<b>Total Stimulus Looking</b>			
Full Sample	$F(2, 66) = 4.30$ $p = 0.02^*$	$F(1, 67) = 1.17$ $p = 0.28$	$F(2, 66) = 0.02$ $p = 0.98$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Smile Half</b>			
Full Sample	$F(2, 66) = 7.48$ $p = 0.001^*$	$F(1, 67) = 0.22$ $p = 0.64$	$F(2, 66) = 0.33$ $p = 0.72$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Face</b>			
Full Sample	$F(2, 66) = 5.44$ $p = 0.007^*$	$F(1, 67) = 0.01$ $p = 0.94$	$F(2, 66) = 0.55$ $p = 0.58$
HR-ASD Excluded Sample	--	--	--
<b>Looking to Internal</b>			
Full Sample	$F(2, 66) = 8.0$ $p = 0.001^*$	$F(1, 67) = 0.01$ $p = 0.92$	$F(2, 66) = 1.93$ $p = 0.15$
HR-ASD Excluded Sample	--	--	--
<b>Eye/Eye+Mouth Looking</b>			
Full Sample	$F(2, 66) = 4.88$	$F(1, 67) = 0.00$	$F(2, 66) = 0.44$

	$p = 0.01^*$	$p = 0.99$	$p = 0.64$
HR-ASD Excluded Sample	--	--	--

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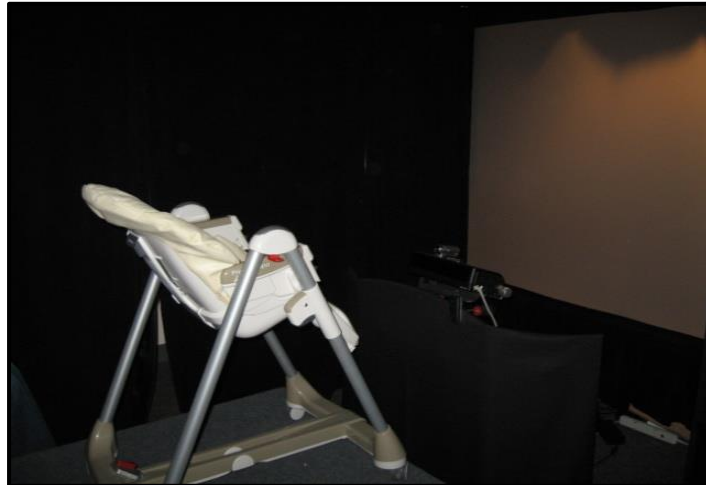
*Note.* \* indicates significance at  $p \leq 0.05$

Table 29. Eye-Tracking Paradigm – Descriptive Data for 6-month-olds Characterized as LR, HR-No ASD, and HR-ASD

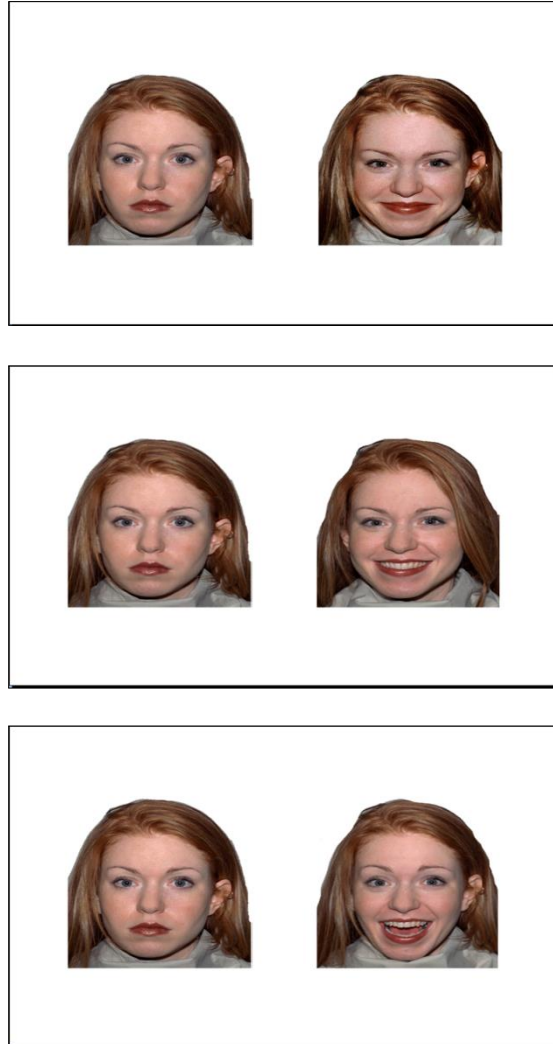
		<b>LR</b> ( <i>n</i> = 20)	<b>HR-No ASD</b> ( <i>n</i> = 17)	<b>HR-ASD</b> ( <i>n</i> = 3)
<b>Total Stimulus Looking</b>	<i>M</i> (SD)	37.34 (20.79)	51.53 (15.39)	56.51 (22.32)
<b>Proportion Looking to Internal Features</b>	<i>M</i> (SD)	0.56 (0.13)	0.67 (0.11)	0.68 (0.18)

*Note.* LR: low-risk, HR: high-risk, ASD: autism spectrum disorder

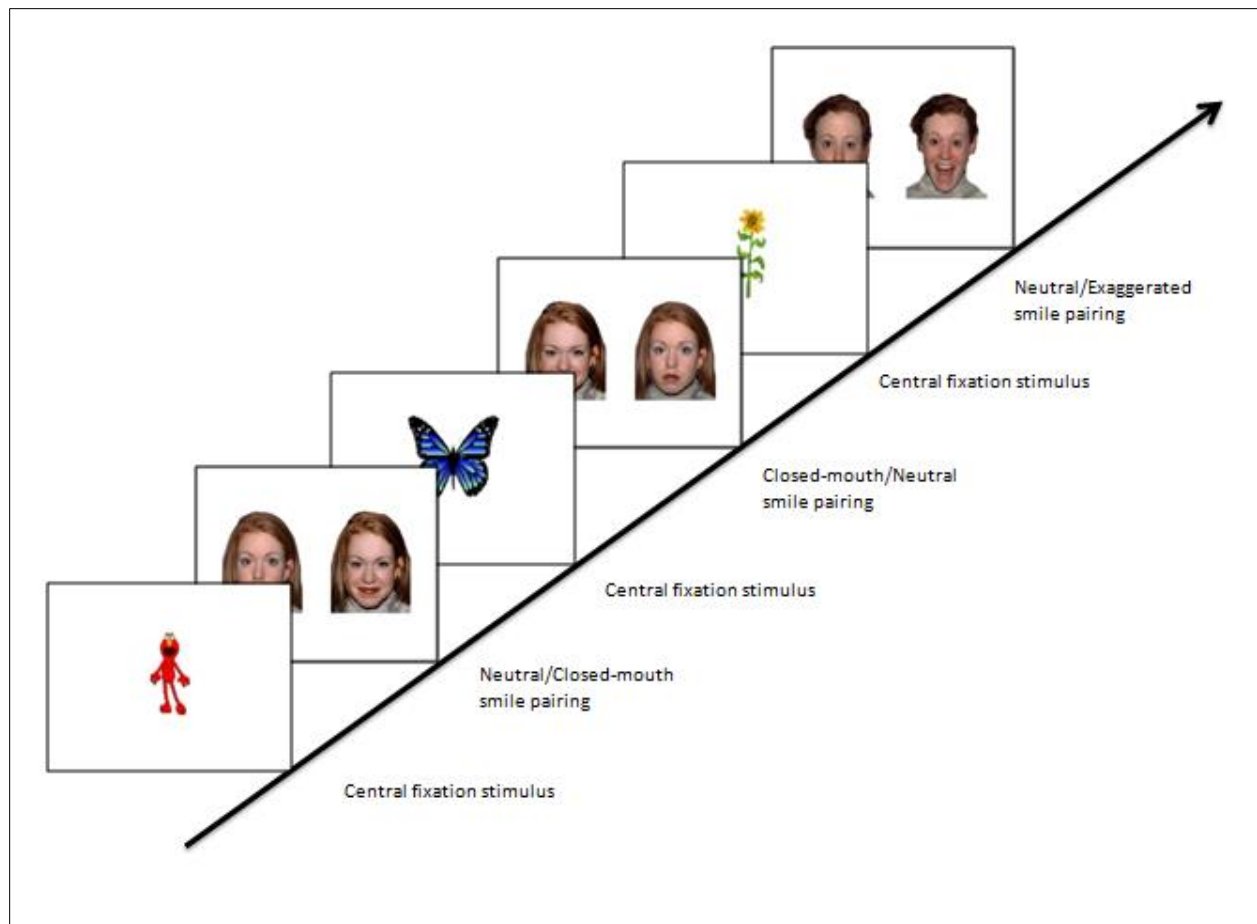
## 6.0 FIGURES



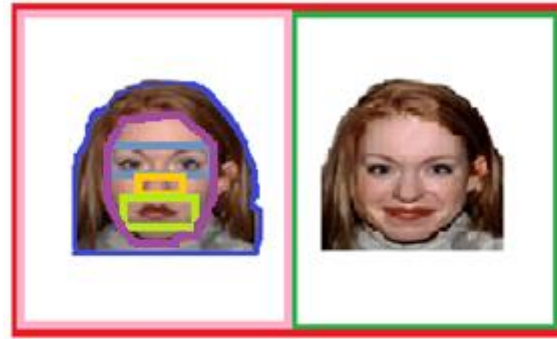
*Figure 1.* Eye-tracking apparatus.



*Figure 2.* The first displays neutral/closed-mouth smile (low intensity), the second neutral/open-mouth smile (moderate intensity), and the third neutral/exaggerated smile (high intensity) stimuli.

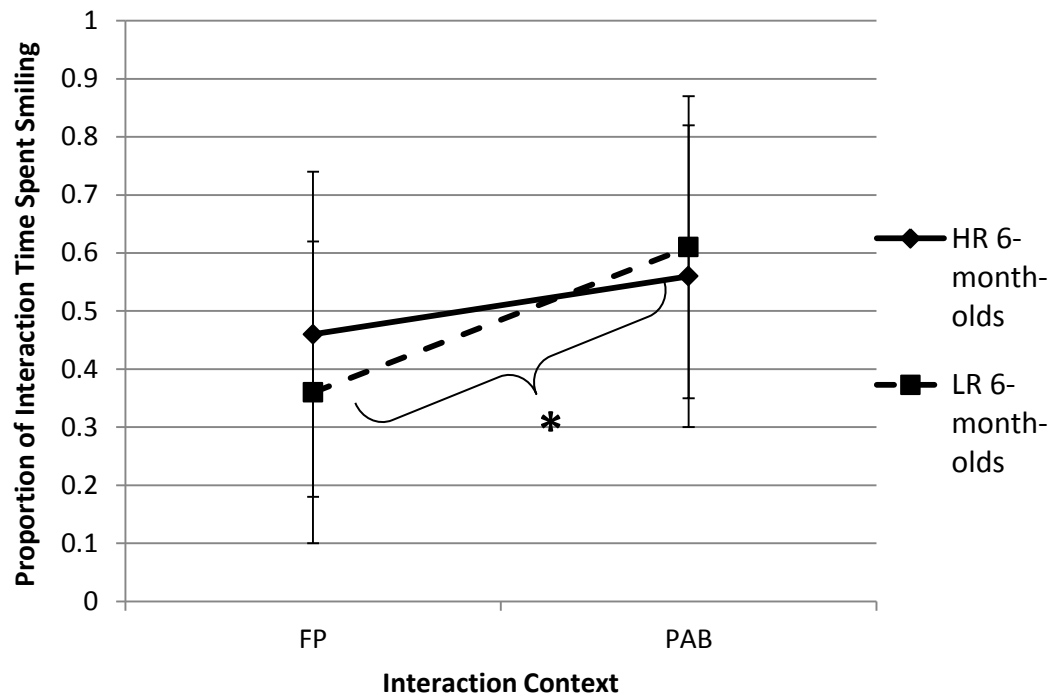


*Figure 3.* A depiction of the eye-tracking visual paired comparison task used to assess emotion perception.



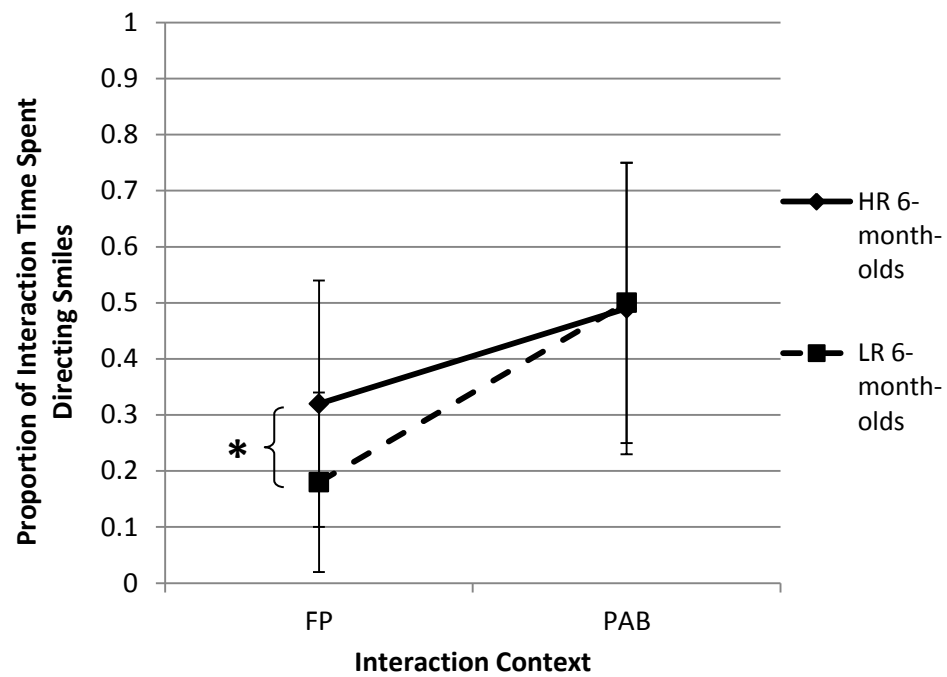
RED: Total stimulus area  
PINK: Neutral side of stimulus  
GREEN: Smile side of stimulus  
PURPLE: Face area  
LIGHT BLUE: Eye area  
ORANGE: Nose area  
LIGHT GREEN: Mouth area

*Figure 4.* A depiction of AOIs used for data reduction and analysis of eye-tracking data.

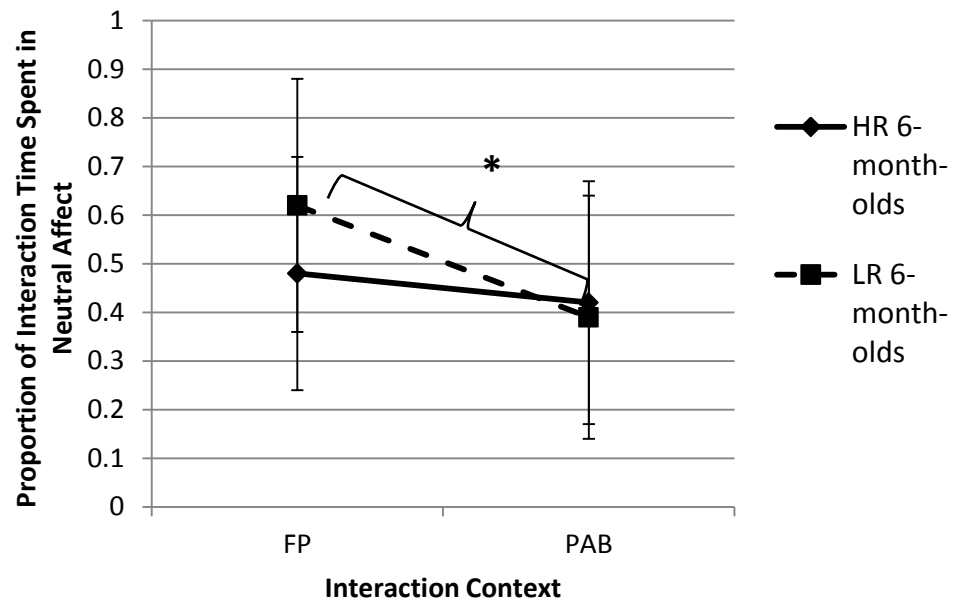


*Figure 5.* Graphical depiction of the proportion of interaction time HR and LR 6-month-olds spend smiling. Error bars represent standard deviations. Asterisks represent a significant risk-group differences at the  $p = 0.00$  level.





*Figure 6.* Graphical representation of the proportion of interaction time HR and LR 6-month-olds spend in directed smiling. Error bars represent standard deviations. Asterisks represent a significant risk group difference at the  $p = 0.01$  level.



*Figure 7.* Graphical representation of the proportion of interaction time HR and LR 6-month-olds spend in neutral affect. Error bars represent standard deviations. Asterisks represent a significant risk group difference at the  $p = 0.00$  level.

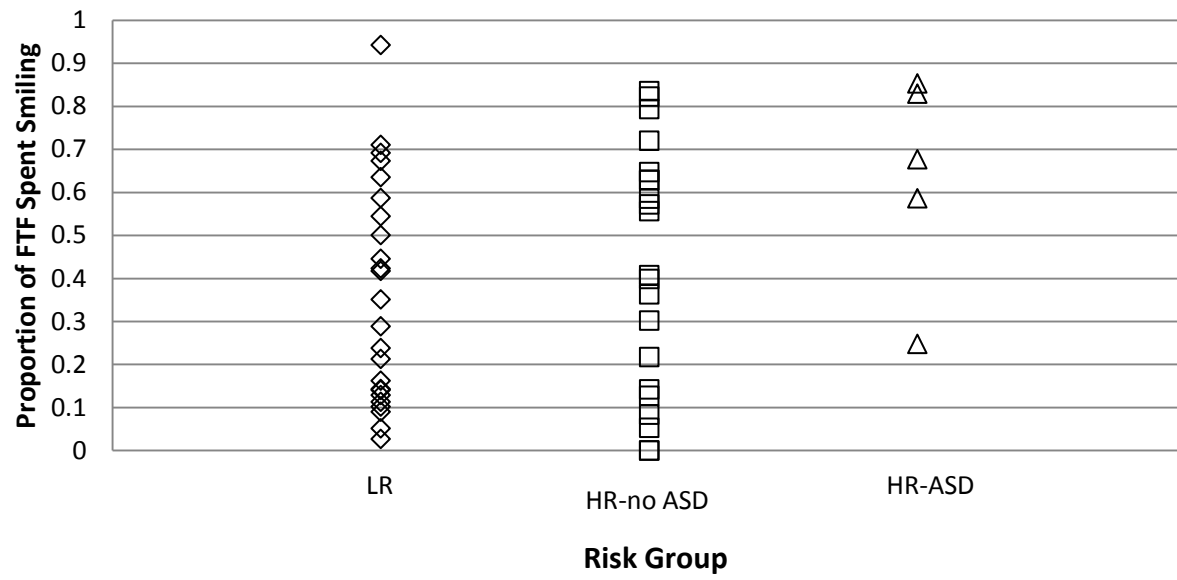


Figure 8. A scatterplot representation of FTF interaction, individual-level, smiling data for LR, HR-ASD, and HR-no ASD 6-month-olds.

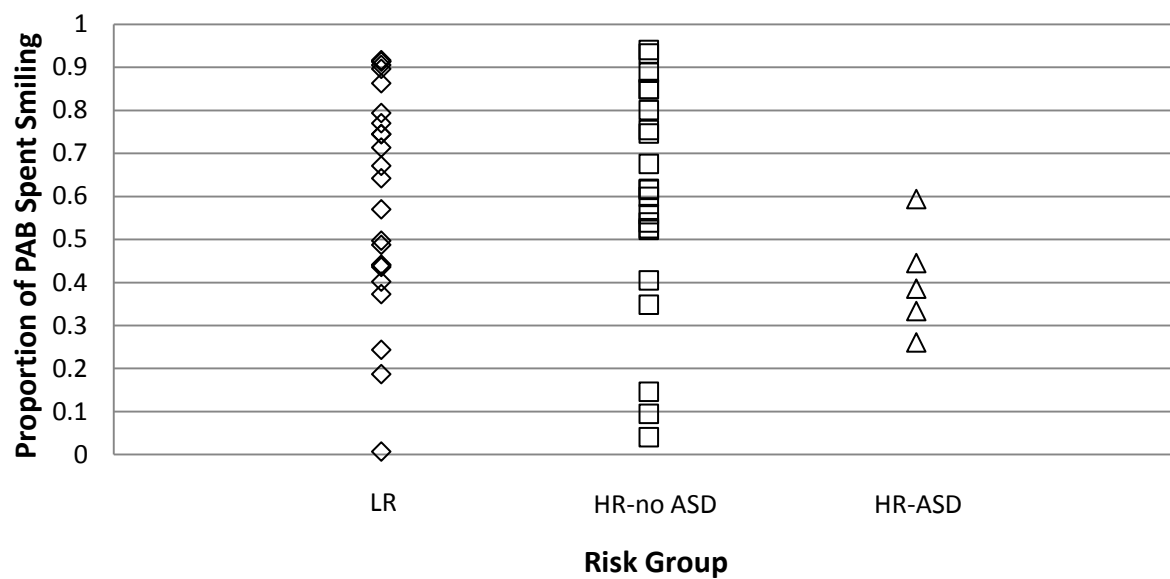
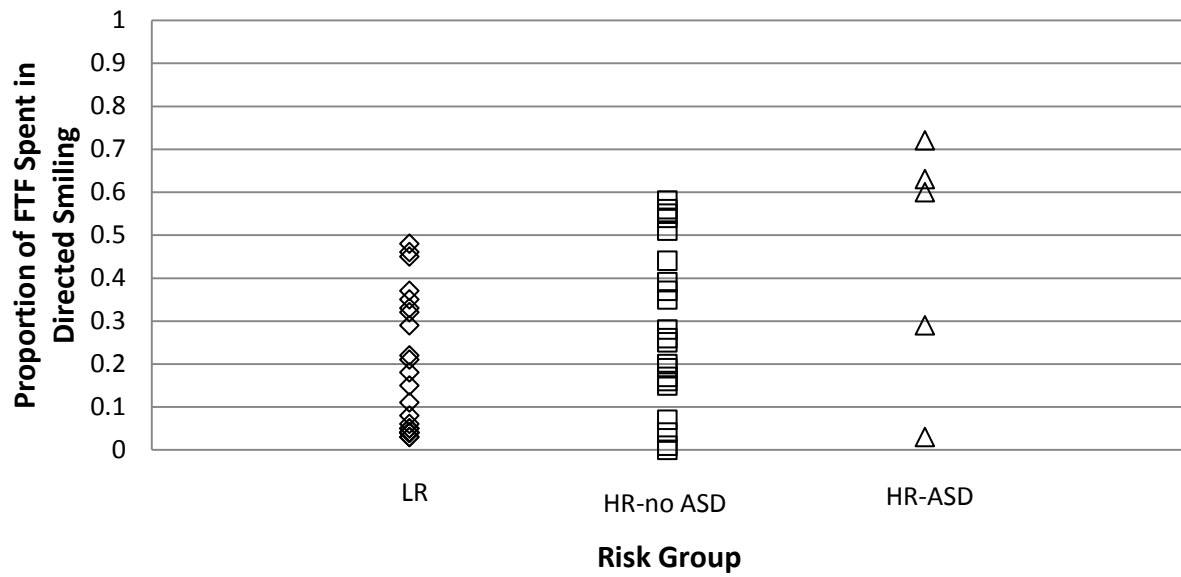


Figure 9. A scatterplot of PAB interaction, individual-level, smiling data for LR, HR-ASD, and HR-no ASD 6-month-olds.



*Figure 10.* A scatterplot of FTF interaction, individual-level, directed smiling data for LR, HR-ASD, and HR-no ASD 6-month-olds.

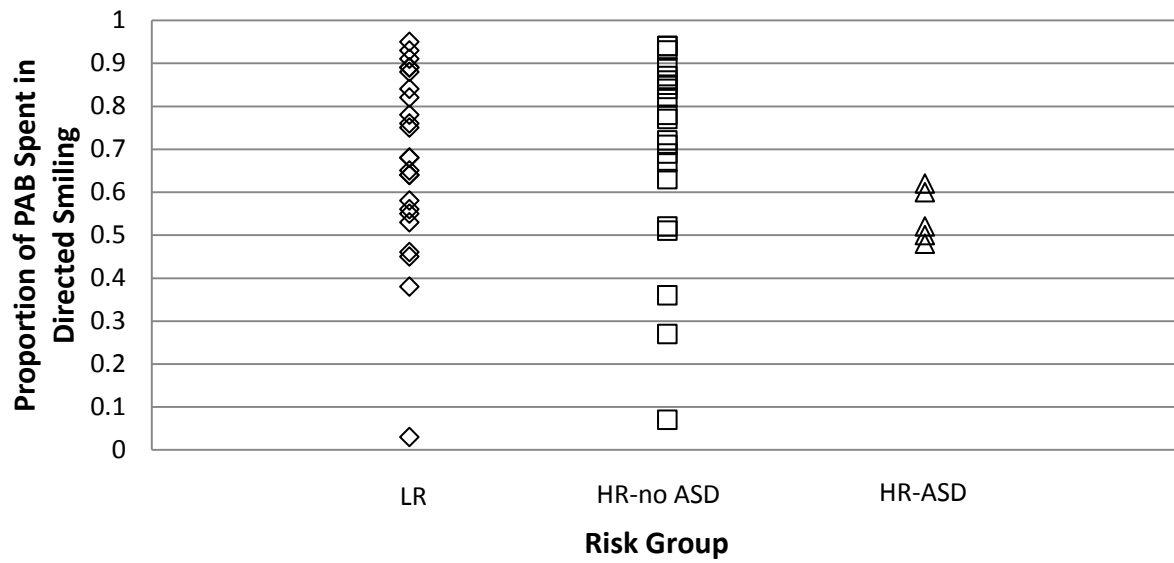


Figure 11. A scatterplot of PAB interaction, individual-level, directed smiling data for LR, HR-ASD, and HR-no ASD 6-month-olds.

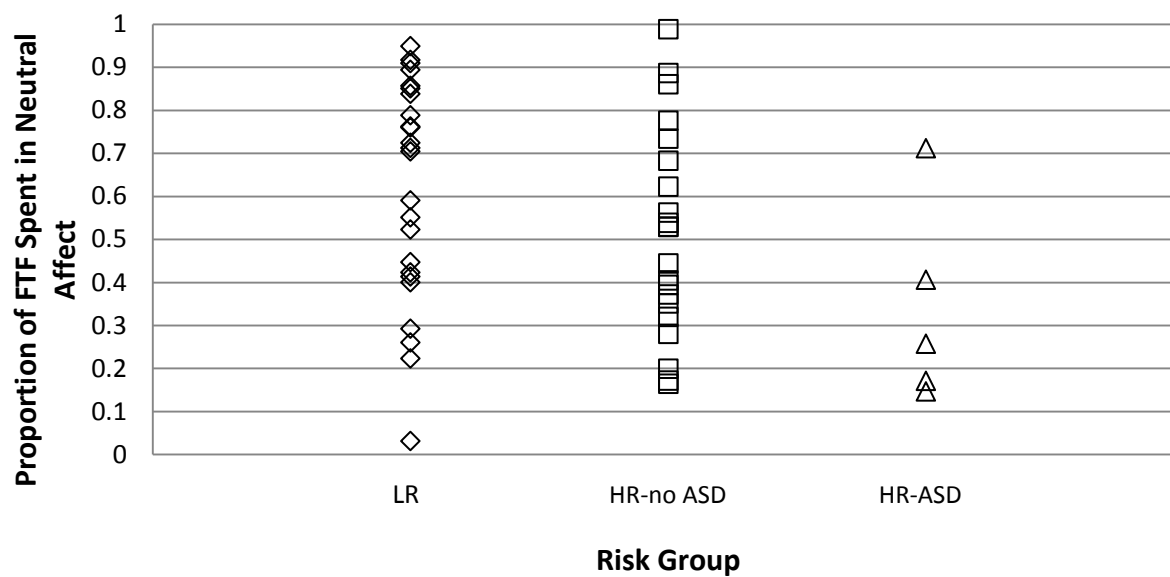
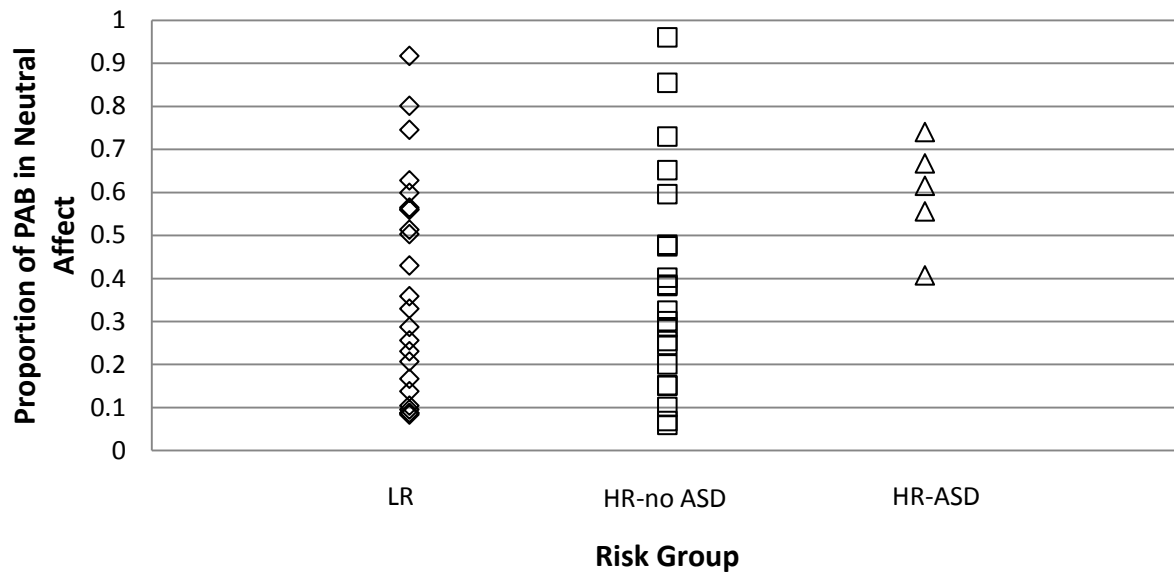


Figure 12. A scatterplot of FTF interaction, individual-level, neutral affect data for LR, HR-ASD, and HR-no ASD 6-month-olds.



*Figure 13.* A scatterplot of PAB interaction, individual-level, neutral affect data for LR, HR-ASD, and HR-no ASD 6-month-olds.



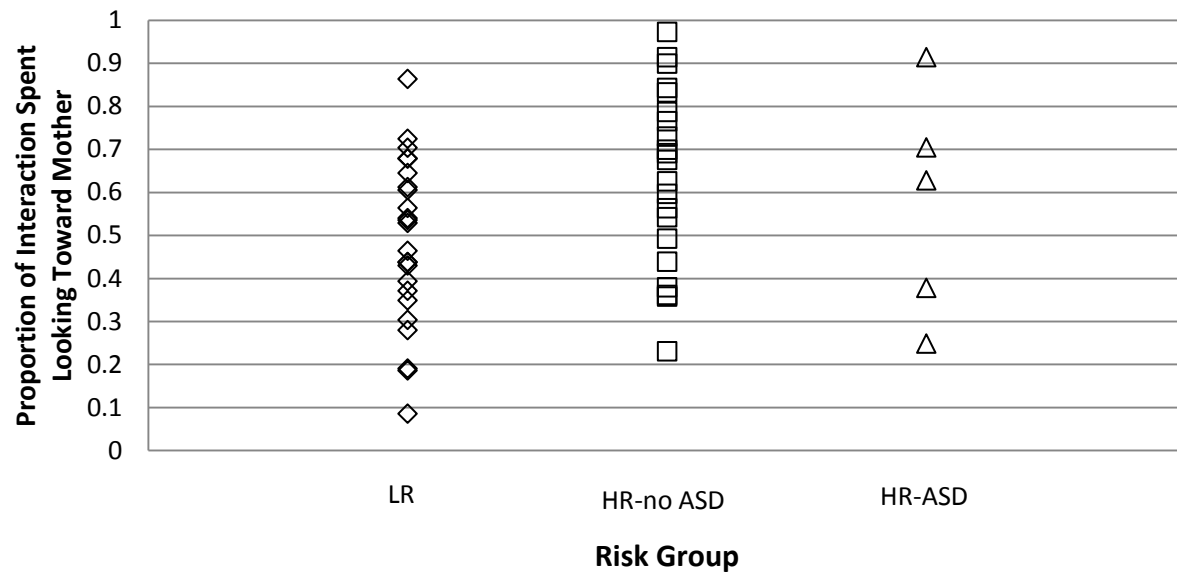
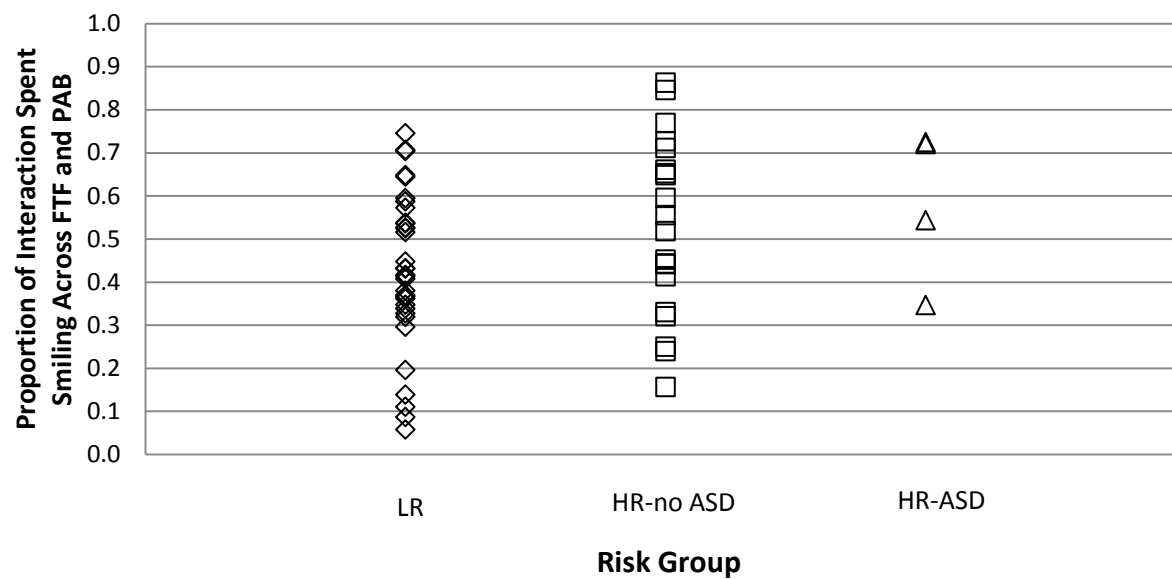


Figure 14. A scatterplot of cross-interaction, individual-level, looking-to-mother data for LR, HR-ASD, and HR-no ASD 6-month-olds.



*Figure 15.* A scatterplot of individual-level smiling data for LR, HR-no ASD, and HR-ASD 11-month-olds.

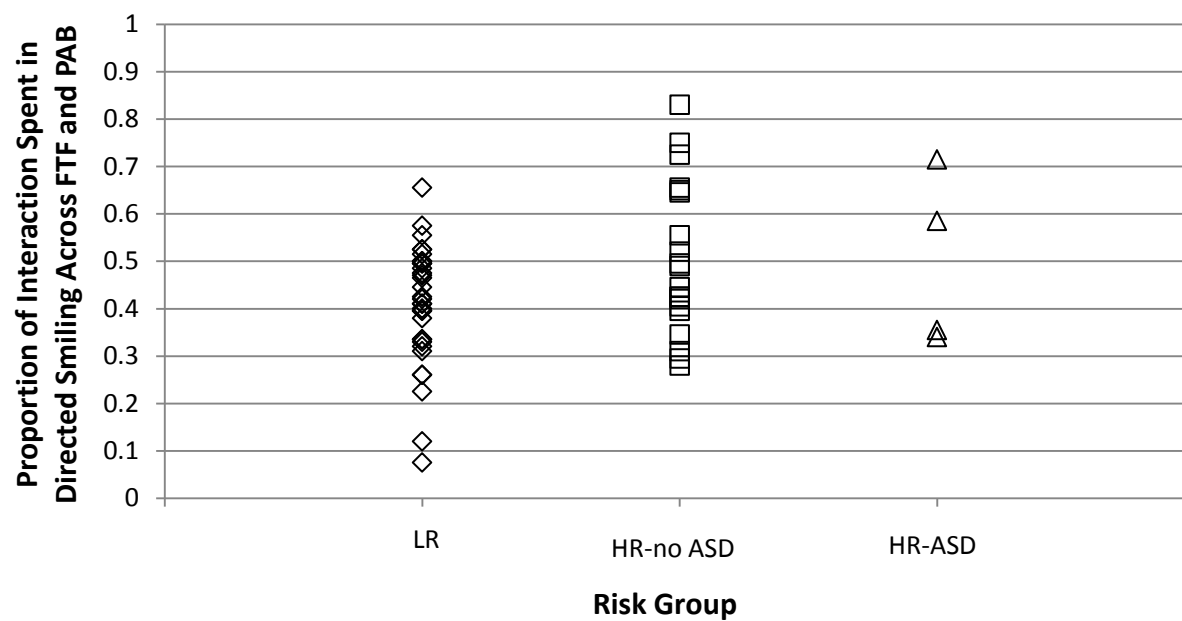


Figure 16. A scatterplot of individual-level directed smiling data for LR, HR-no ASD, and HR-ASD 11-month-olds.

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